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Centre of Marine Environmental Measurements，FIO，SOA

Testing Report

FIO(Ins) [2012] No.: C04-17

Prepared for: Shanghai Cyeco Environmental Technology Co.,Ltd

Test Samples: Environmental parameters, Organisms (>10 μm), Microbes

Test organization: Centre of Marine Environmental Measurements, FIO, SOA

Approval: Xuefen Yin

Issue date: May 5, 2012

Address: No. 6 Xianxialing Road, Qingdao, China

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Centre of Marine Environmental Measurements, FIO, SOA

Cyeco™ BWMS Shipboard Testing Report (300m³/hr capacity)

Number: FIO **(Ins) [2012] No.** **C04-17**

Prepared for	Name: Shanghai Cyeco Environmental Technology Co. Ltd	Contact: Ji Ming
	Address : No.5C, Unit 12, Lane 1097, Pudong Avenue, Shanghai	Tel: 021-58852405
	Sampling date: 2011.10-2012.3	Testing date: 2011.10-2012.4
Samples	Item measured: Temperature, Salinity, Turbidity, pH, TSS, POC, DOC, Organisms (include ≥50 μm and 10 μm ~50 μm), microbes.	Number of measured samples: 192 in total, including 9 for water quality, 45 for 10 μm -50μm organisms, 45 for ≥50 μm organisms, 36 for microbes, 45 for chlorophyll, 12 for TRO.
	Lable: Bottles and membranes with a series of number: S-C-SP- /…和 S-C-SP- /…, etc. **Receiver/sampler**: Yan LI — Sampling time: 2011.10.25-2011.10.27 2012.3.17-2012.3.18 Transfer list ID:201215	Note: Postfix "a" indicating ≥50 μm organisms samples, e.g."S-C1-SP1B/a"; "b" indicating organisms (10 μm -50 μm) samples, e.g. "S-C1-SP1B/b"; "c" indicating microbes samples, e.g. "S-C1-SP1B/c"; "d" indicating water quality samples, e.g. "S-C1-SP1B/d", and on postfix for chlorophyll sample labels, e.g. "S-C1-SP1B"

	Item	**Parameters**	**Standard**	**Methods**	**Equipment/Model**	**Analyst**
Testing	Environ-mental parameters	T, S, pH, NTU,TSS, POC,DOC, TRO	GB/T12763.5-2007, GB17378.4-2007,	T and S equipment pH: Acidmeter Turbidity:spectroph-otomerric method, TSS:weight method; POC and DOC: Combustion method	Multi-parameter water quality instrument; Analytical Balance Elementar analyser TOC-V_{CPH}Aanalyzer 722S Spectrophoto-meter	Xie Linping Li Yan
	Plankton	≥50 μm, ≥10~50 μm, chl-a	GB/T12763.6-2007	Neutral red Staining, count with stereo-microscope;FDA-PI staining,count with invert microscope-fluorometer method	LeicaL2 stereo-microscope NikonTE2000-U invert microscope, Turner fluorometer	Linping Ping SUN Ruixiangli
	Microbes	Bacteria Vibrio spp.; Escherichia coli; Enterococci	GB17378.4-2007 ISO9308-1 :1998 ISO 7899-2 :2000	Plate method, Membrane filter method		Zhangjia
Results	Appendix 1:Results for environmental parameters of the On-Board Testing of Cyeco™ -BWMS Appendix2: Results for organisms (≥50 μm) of the On-Board Testing of Cyeco™ -BWMS Appendix 3:Results for organisms (10 μm~50 μm) of the On-Board Testing of Cyeco™ -BWMS Appendix 4: Results for chlorophyll a of the On-Board Testing of Cyeco™ -BWMS Appendix 5:Results for microbes of the On-Board Testing of Cyeco™ -BWMS					

Tested by	LI Yan	**Checked by**	Qu Lingyun	**Approved by**	Yuefeng Yin
Date of testing	2012.5.5	**Date of checking**	2012.5.5	**Date of approval**	2012.5.5

Conclusion

The shipboard testing of Cyeco^{TM}-BWMS manufactured by Shanghai Cyeco Environmental Technology Co., Ltd was conducted on the cruise ship of Xinjianzhen No.1 from Shanghai-Osaka route during 23~28 Oct.2011and 12-22 March, 2012. Samples were collected and pre-treated on board. According to the testing results and the Guidelines for approval of ballast water management systems- G8 (G8 Guidelines) and Regulation D-2 Ballast Water Performance Standard (D-2 Standard), the conclusion was made as follows:

1. The average water temperature during the first, second and third run was 24.2℃, 23.3℃ and 15.0 ℃ respectively, and the salinity of those was 30.0 PSU, 34.3 PSU and 32.5 PSU respectively. The turbidity was lowest (0.54) at the Kanmon Straits during the second run, and the value was 3.51 at Osaka Bay during the first run and 2.09 at the adjacent area of Yangtze River Estuary during the third run. The TSS was > 20 mg/L during the first run and around 3 mg/L during the other two. The DOC content during the first two run was 1.24 mg/L and 0.57 mg/L, and the POC content was 2.84 mg/L and 1.31 mg/L. The TSS, DOC and POC level in the Yangtze River Estuary area were between the first two run.

The TRO was only tested during the third run. The TRO value varied between 0.062 mg/L-0.066 mg/L, no obvious difference between the control and treatment group of the discharged water. The ultraviolet treatment would not cause the increase of TRO.

2. The dominant organisms ≥50 μm were *Paracalanus sp.*, *Labidocera euchaeta*, *Spoinidae* larva, *Harpacticoida* and *Oithona* sp.. The organisms abundance of this size group in the influent water was high in during the first and second run, with average abundance of 2.54×10^4 ind./m3 and 2.18×10^4 ind./m3 in the two runs. The abundance during the third run was 1.09×10^3 ind./m3 and no living organisms were observed in all the treated groups which meet the criteria of G8 and D-2 Standard.

3. The diatom species were most abundant in the ≥10~50 μm plankton organisms group. The dominant species were different among different runs. In the Japan seas, *Skeletonema costatum*, *Thalassionema nitzschioides*, *Bellerochea malleus*, *Thalassionema frauenfeldii*, *Chaetoceros curvisetus* were the dominant species. In Kanmon Straits, trichodesmium accounted for a large proportion except the species mentioned above. After the third run, the dominant species were *Eucampia zodiacus Pseudo-nitzschia pungens*, *Chaetoceros curvisetus*, *Skeletonema costatum*, as well as some smaller-sized dinoflagellates, Cryptomonas and Chrysocapsaceae etc. The cell density were all excess 10^2 cell/mL in the three runs, with 184.47 cell/mL in Osaka coastal waters and 101.36 cell/mL in Kanmon Straits. The cell abundance in the effluents of the three runs were all in the range of 30~100 cell/mL which meet the requirement of G8. No viable organisms were observed in the effluents of the three runs which met the D-2 standard.

4. The heterotrophic bacteria colony numbers in the influents were in the range of 2.2×10^4 CFU/100mL~5.6×10^4 CFU/100mL, with relatively lower value in Kanmon Straits, and all the results met the standard of G8. The average heterotrophic bacteria colony numbers in the first, second and third run of the effluents were 29.4 CFU/100mL, 24.3 CFU/100mL and 18.3 CFU/100mL respectively. The colony number of *Escherichia coli* in the first and second run of effluents was 22.3 CFU/100mL and 7.3 CFU/100mL respectively. No *Escherichia coli* was dectected in the effluents of the third run. No *Vibrio* spp. and *Enterococci* was detected in effluents of any of the three runs which met the requirement of D-2 standard and G8.

In summary, the treatment effects of the test system to all the size fractions of organisms met the requirement of D-2 standard and G8.

Compiled by	Ruixing Li	**Checked by**	Qu Zhiyun	**Approved by**	Yuefen Yu
Date of compiling	2012.5.5	**Date of checking**	2012.5.5	**Date of approval**	2012.5.5

Cyeco^{TM}-BWMS
(Ballast Water Management System)

Type approval
Shipboard Test Report

(300m^3/h capacity)

Test Organization: First Institute of Oceanography, SOA

Supervising Unit：China Classification Society

Manufacturer：Shanghai Cyeco Environmental Technology Co., Ltd

Testing Site: China-Japan International Ferry Co., Ltd, COSCO
"CHINJIF" Vessel

May. 2012

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1. Introduction

Ships transport 5-10 billion tons of ballast water annually all over the world (Endresen et al. 2004). The ballast water is loaded with particulate sediment and an enormous variety of (living) organisms, which ranges from juvenile stages, larvae and eggs of fish and larger zooplankton (Williams et al. 1988; Carlton & Geller 1993) to macroalgae, phytoplankton (Hallegraeff et al. 1997; Hamer et al. 2000), bacteria and viruses (Gollash et al. 1998).

In general these organisms belong to the natural ecosystem in and around the port of origin but they might not be occurring naturally in the coastal waters and port of destination at the end of a ship's voyage.

In hundreds of cases around the world, this has resulted in severe damage to the receiving ecosystem and to human health, because these non-native organisms developed into a plague. This often has a high impact on the ecosystem and can cause economical damage (Hoagland et al. 2002), as it results in a decrease of stocks of commercially valuable fish and shellfish species and occasionally outbreaks of diseases such as cholera (Ruiz et al. 2000; Drake et al. 2001). If action is not taken, the problem of invasive species will increase in an exponential manner for several reasons.

Ships are getting larger, faster and the amount of traffic across the oceans is expected to increase rapidly during the coming decades, and therefore also the chance of non-indigenous organisms to have large enough numbers for settling and expanding. The problem of invasive species is considered as one of the 4 major threats of the world's oceans next to land-based marine pollution, overexploitation of living marine resources, and physical alteration/destruction of habitats.

To minimize these risks for the future, the International Maritime Organization (IMO) of the United Nations has adopted the Ballast Water Convention in 2004 (Anonymous 2005). The Convention states that finally ALL ships (>50,000 in number) should install proper ballast water treatment (BWT) equipment on board between 2009 and 2016.

As a temporary and intermediate solution for the time being ship may reduce the risk of invasive species by performing ballast water exchange during their voyage when passing deep water (>200 m depth and 200 M from the coast) (Zhang F.Z & M Dickrnan1999). Ballast water exchange faces many problems as to feasibility, safety and efficacy for a large part of ships' voyages the required depth and/or distance to shore requirements are never met; BW exchange can affect the ships construction stability and in rough seas exchange is not possible because of the risk to ship and crew. Treatment of ballast water is therefore considered to be the best solution of reducing the risk of invasive species. During the recent years numerous solutions for treatment of ballast water have been mentioned and tested with the ultimate goal to reduce the amount of organisms in ballast water (Rigby & Taylor 2001). Recently a ballast water management system developed by Hyundai Group of Korea is firstly installed aboard a super crude ship. The company undertook the order from OSC company at 2008， which was the first time that installing a ballast water treatment equipment aboard a super crude ship. （http://twitter.com/yonhapcn）. The ballast water treatment research in China is just at the experimental stage. To develop effective ballast water treatment system could play a great role in protecting Chinese even the whole world's ocean environment and reducing the risk of invasive species.

At the behest of Cyeco Environmental Technology Co., Ltd., we measured the test samples treated by Cyeco^{TM} Ballast Water Management System on-board Xinjianzhen No. 1 on the route of Shanghai-Osaka.

2. Sampling and analyzing methods

2.1 Sampling volume, time and method

The sampling volume and sampling time for various analyses are listed in Table 2.1 and Table 2.2 respectively. Water samples were collected directly from the discharge outlet with 2.5L plastic buckets. The water samples were mixed thoroughly and separated into subsamples for the analysis and pretreatments of different parameters.

Except for DO, samples for water quality testing were collected at discharge outlet directly with 2.5L plastic buckets. The samples were taken to the on-site lab and well mixed, subsamples were then collected for water quality analysis or pre-treatments. For DO, samples were siphoned to brown bottles using a special gastight tubing, which was properly fitted to the sampling outlet of the ballast water simulating tanks.

Table 1.1 Sampling water volume and sampling quantity at different treatment stages

	Influent ballast water(SP1)	Treated tank discharges(SP3)	Control tank discharges (SP4)
Temperature, Salinity	measured at the discharge outlet	measured at the discharge outlet	measured at the discharge outlet
NTU、pH、TSS、DOC、POC	2.5 L×1 ×3	/	/
Organism ≥50 μm	1 m^3 ×1×3	1 m^3 ×3×3	1 m^3 ×1×3
Organism 10 -50 μm	1 L×1×3	1 L×3×3	1 L ×1×3
microbes	500 mL ×1×3	500 mL×3×3	/

※：total sample quantity: 45

Samples for organisms ≥50 μm were filtered through a net with mesh size of 50 μm , diameter of 50 cm at opening and 1 meter length (Fig 2.1). 1 m^3 of sample water was filtered and then transferred to a small plastic bottle with a tag. Samples for the organisms between 10-50 μm were filtered through a net with mesh size of 10 μm, diameter of 20 cm at opening and 25cm length (Fig. 2.2). 1 L of sample water was filtered and then transferred to small bottles with a tag.

Samples for microbes were taken at another outlet of the drainage pipeline directly in order to reduce the contamination by air. The sample bottles were treated under high temperature sterilization before sampling. Disposable gloves were worn and sterile operation was conducted as far as possible when sampling.

Fig 2.1 50 μm filtering net

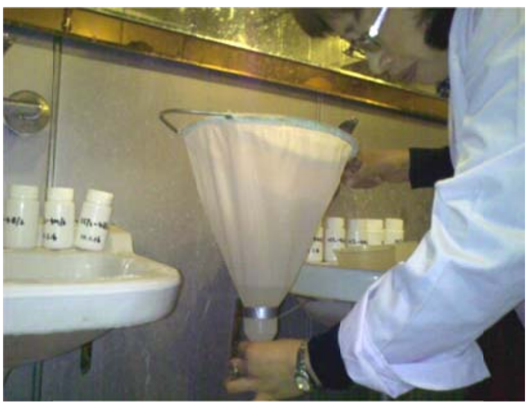

Fig 2.2 10 μm filtering net

Table 2.2. Cyeco^{TM}-BWMS shipboard testing sampling information

Sample types		sample quantity	Sampling location	Sampling date	Sampling time
1st	Influent ballast water （SP1）	3	Osaka Bay	2011.10.25	16:00-16:20 （Tokyo time）
	Treated tank discharges（SP3）	9	Kanmon Straits	2011.10.26	05:22-05:46 （Tokyo time）
	Control tank discharges (SP4)	3	Kanmon Straits	2011.10.26	06:02-06:18 （Tokyo time）
2nd cycle	Influent ballast water （SP1）	3	Kanmon Straits	2011.10.26	06:35-06:55 （Tokyo time）
	Treated tank discharges（SP3）	9	Outside Yangtze River Estuary	2011.10.27	06:09-06:33 （Beijing time）
	Control tank discharges (SP4)	3	Outside Yangtze River Estuary	2011.10.27	06:47-07:03 （Beijing time）
3rd cycle	Influent ballast water （SP1）	3	Outside Yangtze River Estuary	2012.3.17	00:40-00:54 （Tokyo time）
	Treated tank discharges（SP3）	9	Osaka Bay	2012.3.18	19:40-20:03 （Tokyo time）
	Control tank discharges (SP4)	3	Osaka Bay	2012.3.18	19:12-19:21 （Tokyo time）

2.2 The treatment and storage of samples

2.2.1 The treatment and storage of samples for water quality analysis

During the test, a room on the deck floor was emptied as a lab, in which sample analysis or pre-treation would be conducted immediately after sampling. All the samples should be analyzed or pre-treated within 6h after collection. Samples for TSS, POC and DOC analysis were stored in the freezer on the ship and kept in a closed container with ice when transported from Shanghai to Qingdao. The samples were stored immediately at -20 ºC freezer when arrived at Qingdao.

2.2.2 The treatment and storage of samples for biological analysis

For the raw ballast water samples, all the organisms were assumed to be viable. After the sampling, viable organisms (≥50 μm and 10~50 μm) were counted with an inverted microscope and a stereo microscope in the shipboard lab. After the counting, organisms ≥50 μm were fixed with formalin (final concentration 4%), and organisms 10-50 μm were fixed with Lugol's solution (final concentration 2%), and stored under the ambient temperature.

For the biological samples in the discharges, organisms(≥50 μm) were dyed with neutral red and stored at -20 ºC freezer, and transported back to Qingdao with other water quality samples. Organisms (10-50 μm) were counted with an inverted microscope in the control tank and treated tank for the dead and viable cells respectively at the shipboard lab and were taken back to our lab in Qingdao to make further identification and counting.

Samples for microbes analysis must be collected with sterile operation. Sample bottles were treated with high temperature sterilization. Inoculation in the shipboard lab should be conducted immediately after sampling, then the samples would be cultivated at 37 ºC in incubator.

2.3 The methods and guidelines for analysis

2.3.1 Water quality

1） **Temperature and salinity**：Using a Mettler handheld instrument parameters. The salinity meter was calibrated against 0 and 33 PSU standard (sea) water. The accuracy of the salinity measurement is 0.5 PSU.

2） **pH**： pH-metric method，subsamples were measured in-situ using a pH meter.

3) **NTU**: spectrophotometric method. Subsamples were measured in-situ using a spectrophotometer.

3） **TSS**：Weight method. Pre-weighted glass fiber filters are used. Each filter was coded and stored in a clean Petri dish. The filtered volume was dependent on the particle load and concentration and type of organisms present in the water. The higher the total particle load in the sample, the smaller was the volume that could be filtered before the filter clogs. Practical volumes were between 100 and 1000 mL per sample, after filtration the filter was rinsed with fresh water (MiliQ) to remove sea salt. Filters were dried overnight at 60 °C and allowed to cool in a vacuum exicator before weighing. The total amount of suspended solids was calculated from the weight increase of the filter.

4） **POC**：High temperature combustion method，measured with an elemental analyzer. Water samples were filtered over pre-weighted glass fiber with 450°C combustion (the filtered volume was dependent on the particle load and concentration of organisms present in the water), the samples on filters were packed with a aluminium foil, coded, and then saved at -20°C, after the whole test, these samples would be taken back to our lab in QingDao and dried over 12h at 60 °C. The elemental analyzer（ElementarVarioELIII, produced by German）would be used to measure POC.

5） **DOC**：High temperature combustion method，measured with TOC-VcpH analyzer of Japan for analysis. Samples for DOC (15mL) were filtered through GF/C filters and sealed in pre-combusted glass ampoules after adding 50 µl of phosphoric acid (H_3PO4)., saved at -20°C and taken back to our lab in QingDao. Further measurement was conducted after samples were defrosted to room temperature. Standards were prepared with potassium hydrogen phthalate.

6）TRO： Principles: enough I^- was added to samples before measured, in the acidic conditions (pH of 3.0- 4.0), the residual oxidants of samples would oxidize I^- to I_3^- or I_2 which were lightbrown and soluble. Then read the absorbance of spectrophotometer at the wavelength of 353 nm. At last, determine the TRO concentrations of the samples according to the standard curve, the unit of TRO concentration was equivalent concentration (μeq./L) or equal to Cl_2 concentration (mg/L as Cl_2).

Sample Collection: Collect sample waters with dissolved oxygen bottles of 60mL, the overflow water volume should be 3 – 4 times of bottle volume (avoid the generation of bubbles), 0.5 mL of buffer and 0.5 mL of KI solution were added and then closed the tap, reverse the bottle over several times to mix water samples uniformLy, after which put the bottles into a plastic box with tap, took them back to the on-site lab for measurement after all the samples were collected

Procedure for Determining：

(1) open the sample bottle, read the absorbance of spectrophotometer（ABS_{raw}）at the wavelength of 353 nm within 10 minutes to 2 hours after adding the reacting solution.

(2) Blank

Add deionized water into dissolved oxygen bottles of 60mL, determine the absorbance of blank sample（ABS_{blank}） as the normal procedure of determining. Generally, the ABS_{blank} was below 0.002ABS.

(3) Turbidity background

0.5mL sodium hyposulfite was mixed with the remaining samples to eliminate the color of iodine, then determined again to get the absorbance(ABS_{turb}）of background sample.

(4) Preparation of the standard curve

a) Prepare 100 mL standard solution by diluting 1.0mL of potassium permanganate standard solution with deionized water, then prepare standard solutions in five gradient of concentration ranged from 0 to 100 μeq. / L with the former solution, similarly, diluted to 100 mL with deionized water.

b) The standard solutions were added to 60mL of dissolved oxygen bottles, with the procedure of (1) and (2), the slope (L/μeq.)of standard curve was obtained.

（5）Data processing：

a. Calculate corrected absorbance values of samples by subtracting the absorbance of this water specific blank and turbidity background from the samples:

$$ABS_{corr} = ABS_{raw} - ABS_{blank} - ABS_{turb}$$

b. Use the slope of the standard line and the corrected value determined from the calibration to determine the TRO concentrations of the samples.

$$C\ (\mu eq.\ /\ L) = ABS_{corr}\ /\ S$$

Where:

C ： Equivalent concentration (μeq./L) of TRO in water samples

S ： slope of the standard curve。

Theoretically, the unit of TRO was μeq/L, however, conversion to unit of Cl_2 concentration was more common for easy analysis:

$C(mg/L\ as\ Cl_2) = C(\mu eq.\ /\ L) \times 71\ /\ 1000$

2.3.2 Plankton

The majority of the large size fraction (>50 μm) consists of zooplankton, while the majority of the small size fraction (10-50 μm) consists of phytoplankton. Samples were filtered by a 50 μm and a 10 μm net respectively （volume of filtered water is shown on Table 2.1）. Then it was concentrated to 150 mL and poured into a small plastic bottles , wash the sieve twice and transfer the flushing fluid to the plastic bottles together, the samples for human pathogens analysis were taken in sterile sealed bottles.

1）**Organisms ≥ 50 μm**

After sampling，identification and counting of viable organisms were taken with a stereo microscope before fixation. If the density of viable organisms was high, subsamples was taken with a quantified sampling tube or a sample splitter which can separate the sample into equal subsamples. Then one of the subsamples was analyzed. The observation on organisms' activities was taken under microscope at 20-160x magnification. The results of identification and counting were recorded. When the counting of viable organisms was finished, formalin solution (the last concentration is 5%) was added to fix the samples. A further identification and counting of total amount of organisms was conducted after the samples were taken back to Qingdao. Then number of individuals per cubic metre was calculated.

The equation for abundance of organisms is as follows：

$$C_B = \frac{N_B}{V}$$

where：

C_B——density of zooplankton per volume，unit（ind./m^3）;

N_B——total number，unit（inds or cells）;

V——the volume filtered ，unit（m^3）.

2）**Organisms** 10-50μm

It is difficult to count all the organisms for 10～50μm fraction. A practical method is to adjust the concentration of the cells to a certain value. Then 1mL of well-distributed sample were randomLy taken and counted with a counting chamber. The observation on organisms' status was made with a invert microscope at the field lab. The results of identification and counting were recorded. When the counting of viable organisms was finished, Lugol's solution (the last concentration is 1%) was added to fix the samples. A further identification and counting of total amount of organisms was conducted after the samples were taken back to Qingdao. Then number of cells per milliliter was calculated

The equation is :

$$C = \frac{n \cdot V_1}{V_2 \cdot V_n}$$

where：

C——organisms number per volume of sea water unit（cells/L）;

n——organisms number of one counting unit（cells）;

V_1——sample volume after concentrated，unit（mL）；

V_2——sample filtered over small sieve，unit（L）;（influent water of control 1L，treated water at discharge 10L）

Vn——sample volume for counting，unit（mL）（we have two kind of counting chamber : 1mL and 0.5 mL）

2.3.3 Analysis of human pathogens

Inoculation should be taken within 2h after sampling. Count the number of colonies according to the international standard.

1) Heterotrophic bacteria：Plate method

Principles:

After incubation of a sample, the dispersed bacteria will develop into isolated colonies. A visible colony on solid medium represents one bacterial cell. The number of heterotrophic bacteria is obtained by counting the number of colonies. The key of this technique is to disperse the heterotrophic bacteria completely and to dilute bacterial sample to several solutions with different concentration. Small volume of diluted solution (containing 100 to 200 cells or less) is spread evenly over the surface of the solid medium.

Procedures:

1 mL Tween solution was added to 100 mL sample. The sample was well mixed to separate the organisms and kept them separated. Take 1mL of the sample with a sterile pipette to a test tube filled with 9 mL of disinfected sea water. After a thorough mixing, 0.1mL of solution was taken and inoculated on the surface of solid medium（2216E）in a Petri dish. Then it was spread evenly with a sterile, L-shaped glass rod. The dish was incubated at 25 ºC for 7d, and then it was taken out for counting the number of colonies.

2) *vibrio cholerae*：Plate technique

The total amount of vibrio is one of the most important parameter for indicating water pollution levels of human pathogens. TCBS selective medium is chosen to examine the amount of vibrio. After the inoculation to the medium in a dish, the dish was incubated for a certain time under optimal conditions. Then the vibrio colonies were counted.

Procedure：

1mL of sample was pipette with sterile operation and inoculated into a test tube with BTB medium solution. It was incubated for 18h at 37 ºC. The bacterial solution shown a positive reaction was taken and lined on TCBS plate, which will be cultivated for 18h at 37 ºC. Check the number of colonies with characteristics of vibrio.

3）*Escherichia coli*： membrane filter technique

The water sample was filtered through a membrane filter. After filtration, the heterotrophic bacteria were on the membrane. Then the filter was placed on a selective solid medium and there should be no entrapment of air. After incubation, the *E. coli* colonies on the membrane were identified and counted. The number of *E. coli* per liter sea water was then worked out.

procedure：

100 mL of sample water was filtered through an acetates membrane with pore diameter of 0.2 μm. After filtration, the heterotrophic bacteria were remained on membrane. The membrane was placed on the surface of a solid medium (M-TEC) without any entrapment of air. After 0.5 h cultivation with the plate inverted in an incubator at 37 ºC, it was transferred to another incubator with 44 ºC for a continuous cultivation of 18-24h. The *E. coli* colonies on the membrane were counted and identified. The number of *E. coli* per liter sea water was then worked out.

4) *Intestinal enterococci*：membrane filter technique

PSE agar plate with selective culture medium is chosen to test the total number of *Intestinal enterococci*. After inoculation, the plate is cultivated in an incubator at 37 ºC for a certain time. The bacterial colonies with characteristics of intestinal enterococci were counted. The colonies may be isolated and purified for further identification. The procedure is the same as that for *E. coli.*

2.3.4 Chlorophyll a

Samples were filtered through GF/F fiberglass membranes and wrapped up with aluminum foil, saved at -20 ºC after marked until measured. Before determined, the samples were first put in a scintillation vial, then we added acetone solution (the concentration was 90%), extracting for over 12hs under cold condition, after which the samples could be measured with the Turner Fluorometer. The concentration of Chl-a was calculated as follow:

$$\rho_V(chl\text{-}a)=\frac{Fd\cdot(Rb-Ra)\cdot V_1}{V_2}$$

Where：

$\rho_V(chl\ a)$ — Chla concentration of sea water. Unit: mg/m^3；

F_d—Conversion coefficient（obtained from the standard curve），unit:mg/m^3；

R_b — fluorometer reading before acidification；

R_a — fluorometer reading after acidification；

V_1 — extract volume，unit（cm^3）；

V_2 — filtered sample volume，unit（cm^3）.

2.3.5 Guidelines and Specifications followed

1) Guidelines for approval of ballast water management systems (G 8) Resolution MEPC. 174（58）
2) Supplementary guidelines for approval of ballast water management systems (G 8) Resolution（BLG 15/5/4，2010）
3) The specification for oceanographic survey - Part 5: Chemistry（GB/T12763.5-2007）
4) The specification for oceanographic survey -Part 6: Biology （GB/T12763.6-2007）
5) The specification for marine monitoring-Part 4: Water quality monitoring and analysis（GB17378.4-2007）
6) The specification for marine monitoring—Part 7: Ecological survey for offshore pollution and biological monitoring（GB17378.7-2007）
7) The methods for determining Total Residual Oxidants (TRO) in sea water－spectrophotometric method⁄spectrophotometric of odine. Taiwan Central

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Table 2.3 Summary of parameters, method, sensibility and guidelines of the test

Parameters	unit	MDL	Method of analysis	sensibility	Guideline
Temperature	℃	NA	Thermometer	0.1℃	specification for oceanographic survey
Salinity	PSU	1.0	Salinimeter	0.1～0.2 PSU	specification for oceanographic survey
pH	pH	0.0	pH-metric method	0.01 pH	The specification for marine monitoring
DO	mg/L	0.1 0.2	winkler method	0.05 mg/L	The specification for marine monitoring, specification for oceanographic survey
NTU	NTU	0.1	spectrophotometric method	0.1 NTU	specification for oceanographic survey
DOC	mg/L	0.36	high temperature combustion method	mg/L	The specification for marine monitoring
POC	mg/L	0.1	high temperature combustion method	mg/L	The specification for marine monitoring
TSS	mg/L	1.0	Weight method	mg/L	specification for oceanographic

					survey
TRO	ueq/L ，mg/L as Cl		spectrophotometric method		Bulletin of Taiwan Environmental Protection Agency
Organisms ≥50 µm	ind/ m^3	1.0	Filtered and condensed with 50 µm sieve ，count with microscope		specification for oceanographic survey
Organisms 10-50µm	cells/mL	1.0	Filtered and condensed with 10 µm sieve ，count with invert microscope		Hallegraeff.G.M ，D.M. Anderson and A.D. Cambella
Chlorophyll a	mg/L		Fluorometer		
heterotrophic bacteria	CFU/100 mL	1.0	Plate method	CFU/mL	The specification for marine monitoring
E.coli	CFU/100 mL	1.0	Filter membrane method	CFU/mL	The specification for marine monitoring
Intestinal enterococci	CFU/100 mL	1.0	Fecal Streptococcus and Enterococcus group	CFU/mL	Standard Method 9230/ MM-FS-CNJ-0351 or ISO4833-2003
vibrio cholerae	CFU/100 mL	1.0	Plate method	CFU/mL	The specification for marine monitoring

2.4 Quantity control

2.4.1 Measures for quality assurance

2.4.1.1 Measures of sampling at test site for quality assurance

All samples were collected at the test site. The water samples were distributed into bottles with tags or labels. To avoid or reduce contamination, the sample bottles were cleaned with hydrochloric acid (samples for pH measurement were not included), then washed with pure water at last twice. Before sampling, the bottles were washed twice again with the sea water of test site. The sample bottles for microbes were autoclaved. The culture medium for microbes incubation were prepared in the lab. Before the test, they were disinfected at the test site. Small plankton nets with 50μm and 10μm mesh size were used for filtering the organisms (>50μm) and the organisms (10～50μm) respectively. After that, the samples were concentrated and transferred into small sample bottles.

2.4.1.2 Measures of storage and transport of samples for quality assurance

During the operations of filtration and distribution of samples, measures against contamination were adopted. When collecting sample for POC, DOC and microbes, it is required to wear gloves. The samples, such as Chl-a, DOC, and POC cannot be analyzed at the test site. They were stored under frozen conditions after pre-treatment. During transportation, they were in a cooler with dry ice. Plankton samples were fixed and the sample bottles were sealed. Then they were taken back to the lab in Qingdao for further analysis.

2.4.2 Quantity control

2.4.2.1 Quantity control of analysis

- All analytical equipments used must meet the requirements in the test，the 722 spectrophotometer, pH meter and electronic balance etc., were all examined by legal authority designated by state, equipments, such as microscopes and fluorometer, must have Calibration report.
- The samples need to be carefully checked prior to analysis and to confirm the

samples are kept well. The inside and outside labels must coincide with the records taken during the test.

- Equipment must be still in normal condition after the analysis.
- When abnormal results occurred, the causes should be found out in time, and explanation and correction should be made. There is a need to repeat the analysis if necessary.
- Except for postgraduate students, all of the personnel conducting measurements and analysis should be qualified to do marine environmental monitoring with certificate. The students have to take in special technical training and their work must be supervised.

2.4.2.2 Quantity control during the test

- A technical introduction and work allocation about the test will be given to all participating personnel. Everyone must clearly understand his/her responsibility for work and results.
- The equipments should be checked as soon as they were moved to the test site to examine whether everything is OK. Another check was conducted when the equipment was set up to examine whether it runs normally. The equipment will be calibrated if necessary. All these activities will be recorded.
- All samplings and analysis should follow relevant valid version of standards, guidelines and specifications.
- The equipment will be checked when all work are finished. It should be in normal condition.
- If the analysis was interrupted or some changes of sampling or analysis have to be made, it should be reported first to the leader of the test. The work could be continued only if it was approved.

2.4.2.3 Quantity control of equipments used

All the equipments were examined by legal authority designated by state. The allowance should be still valid. If the equipment needs only self-examination, it should be examined by relevant experts prior to the test.

2.4.3 The raw records

1) The raw records reflect the exact results of sampling and analysis. Any changes and deletion of them is strictly prohibited. The raw records of sampling have to be checked by the supervisor from Shanghai Branch, China Classification Society with his/her signature at the test site.

2) Tables with unified format should be used for taking the raw records. The use of pencil was not allowed except there is a special definition. The tables should be filled out completely with signature of the analyzer and proofreader.

3) The determination of significant digits and data processing of the raw data should strictly follow the relevant definition in the National standards of China --The Specification for Oceanographic Survey (GB/T12763-2008)and The Specification for Marine Monitoring GB17378.7-2007）

3. Results

3.1 Water quality

Only the water quality in the influent ballast water was measured. The average temperature and salinity of the first cycle was 24.2 ℃ and 30.0. During the second cycle at Kanmon Strait, the ballast water temperature was 23.3 ℃ and the salinity was 34.3 which was close to the oceanic water. The average temperature and salinity of the third cycle was 14.97 ℃ and 32.53 in March, 2012.

Turbidity and TSS concentrations were relatively higher in the coastal waters (Table 3.1). During the first and third cycle, the turbidity was 3.51 and 2.09, respectively and the TSS concentration was 23.5 mg/L and 3.95 mg/L, respectively. During the second cycle at Kanmon Strait, the seawater was more transparent, and the turbidity and TSS concentration was 0.54 and 3.01 mg/L. The pH value was stable among the three cycles and varied between 7.97 -8.15. The POC concentration was highest at Osaka Bay (2.84 mg/L) and lowest at Kanmon Strait (1.31 mg/L). The DOC content was similar to that of POC.

TRO was only measured in the discharges during the third cycle. The TRO was very low and showed on difference between the treated and control discharges (0.062-0.066

mg/L), which suggested the UV treatment would not increase the TRO.

Table3.1 Shipboard testing results of water quality parameters of Cyeco™ BWMS

First cycle Ballast at Osaka Bay（2011.10.25）— Discharge at Kanmon Strait (2011.10.26）		
Parameters	Influent ballast water	
	mean	range
T（℃）	24.2	23.9-24.6
S	30.0	29.9-30.1
turbidity（NTU）	3.51	2.98-3.86
pH	7.99	7.97-8.00
TSS（mg/L）	23.50	21.75-24.5
POC（mg/L）	2.84	2.36-3.39
DOC（mg/L）	1.24	1.04-1.43
Second cycle Ballast at KanmonStrait（2011.10.26）— discharge at outside of Yantze River Estuary（2011.10.27）		
T（℃）	23.3	23.2-23.5
S	34.3	34.0-34.6
turbidity（NTU）	0.54	0.48-0.61
pH	8.14	8.13-8.15
TSS（mg/L）	3.01	2.57-3.69
POC（mg/L）	1.31	1.23-1.45
DOC（mg/L）	0.57	0.44-0.71
Third cycle Ballast at outside of Yantze River Estuary（2012.3.17）—dicharge at outside of Osaka Bay（2012.3.18 ）		
T（℃）	14.97	14.1-16.2
S	32.53	32.4-32.7
Turbidity（NTU）	2.09	1.40-3.42
pH	8.04	8.03-8.05
TSS（mg/L）	3.95	3.00-5.31
POC（mg/L）	1.39	1.12-1.78
DOC（mg/L）	1.09	0.96-1.23

（TRO）mg/L(as Cl)		Control 0.062 (0.062-0.063)	Treated0.065 (0.064-0.066）

3.2 Organisms ≥ 50 µm

Organisms (≥50 µm) were mainly zooplankton. The majority species were belong to the copepod and dominated by *Paracalanus* sp., *Labidocera euchaeta*,late Nauplius larvae,*Harpacticoida* sp. and *Oithona* sp..

During the first and second cycles in Autumn, 2011, although the ballast water took from different area, the zooplankton (≥50 µm) compositions and individual abundances were similar which was 2.54×10^4 inds/m^3 and 2.18×10^4 inds/m^3, respectively. The zooplankton individual abundances were 1.17×10^4 inds/m^3 and 1.23×10^4 inds/m^3 at the control discharges, which decreased about a half compared with the ballast waters. The zooplankton individual abundances were lowest at spring 2012 at the outside of Yangtze River Estuary, which was one order of magnitude lower than that at the first two cycles. The average abundance in the influent ballast water was 1.09×10^3 inds/m^3 and decreased to 215 inds/m^3 during the control tank discharge. No viable organisms was observed in all of the 27 samples from the three cycles and the dead individual varied between 6~72 inds/m^3.

Table 3.2 Shipboard testing results of viable plankton ≥50 µm abundance in the Influent ballast water and discharges of the Cyeco^{TM} BWMS

First cycle Ballast at Osaka Bay（2011.10.25）— Discharge at Kanmon Strait (2011.10.26）				
Parameters	Influent ballast (n=3)	Discharges		
		Control tank discharges (n=3)（viable）	Treated tank discharges (n=9)	
			dead	viable
average density（ind/m^3）	2.54×10^4	1.17×10^4	42	None
ranges	1.70×10^4-3.08×10^4	7.21×10^3-1.50×10^4	27-72	None
Second cycle Ballast at Kanmon Strait（2011.10.26）— Discharge at outside of Yantze				

River Estuary（2011.10.27）				
total density（ind/m^3）	2.18×10^4	1.23×10^4	27	None
range	2.10×10^4-2.24×10^4	7.40×10^3-1.42×10^4	6-57	None
Third cycle Ballast atoutside of Yantze River Estuary（2012.3.17）—Discharge at outside of Osaka Bay（2012.3.18）				
average density（ind/m^3）	1.09×10^3	2.15×10^2	19.1	None
ranges	8.69×10^2-1.33×10^4	0.79×10^2-3.32×10^2	6-29	None

3.3 Organisms ≥10μm ~50 μm

The organisms in this size range were mainly composed of phytoplankton and protozoa. The phytoplankton species composition was slightly different in the first two cruises in Autumn, 2011. The dominant species in Osaka Bay were *Skeletonema costatum*, *Thalassionema nitzschioides*, *Bellerochea malleus*, *Thalassionema frauenfeldii* and *Chaetoceros curvisetus*. During the second cycle in Kanmon Strait, the dominant species were not significant, and *Trichodesmium* sp. contributed the largest proportion besides *Skeletonema costatum, Bellerochea malleus* and *Chaetoceros curvisetus*. During the third cycle at the outside area of Yangtze River Estuary, the dominant species were *Eucampia zodiacus, Pseudo-nitzschia pungens, Chaetoceros curvisetus* and *Skeletonema costatum.* Besides, some smaller sized algae such as dinoflagellates, Cryptophyta and Crysophyta were commonly detected.

The average phytoplankton cell abundance in the first, second and third cycle was 184.47 cell/mL, 101.36 cell/mL and 104.42 cell/mL, respectively. The reduction of cell abundance in the control tank discharges was not significant due to the short time interval (about one day) between the ballast and discharge water (Table 3.4). No viable phytoplankton was detected in the treated tanks.

Table3.3 Dominant phytoplankton species ≥10 μm~50 μm of shipboard testing of Cyeco^{TM} BWMS

Species	Phylum	Osaka Bay	Kanmon Strait	Outside Yangtze River Estuary
Skeletonema costatum	diatom	++++	+	++
Thalassionema nitzschioides	diatom	+++		
Thalassiothrix frauenfeldii	diatom	++	++	
Chaetoceros curvisetus	diatom	++		+++
Bellerochea malleus	diatom	++	+++	
Trichodesmium spp.	blue-green alga		++++	
Eucampia zoodiacus	diatom			++++
Other flagellates	main dinoflagellate			++++
Pseudo-nitzschia pungens	diatom			+++
Thalassiosira sp.	diatom			++
Other flagellates(Crysophata?)	main golden alga	+++		

Table 3.4 Viable phytoplankton ≥10 μm~50 μm cell abundance of shipboard testing of CyecoTM BWMS

First cycle Ballast at Osaka Bay（2011.10.25）— Discharge at Kanmon Strait (2011.10.26）				
Parameters	Influent ballast water (n=3)	Discharges		
		Control tank discharges (n=3)（viable）	treated tank discharges(n=9)	
			dead	viable
average abundance（cell/mL）	184.47	111.88	6.23	None
ranges	172.25-200.67	98.96-122.29	3.13-7.90	None
Second cycle Ballast at Kanmon Strait（2011.10.26）— Discharge at outside of Yantze River Estuary（2011.10.27）				

total abundance（cell/mL）	101.36	31.28	3.50	None
ranges	97.52-107.50	29.50-34.17	2.15—4.44	None
Third cycle Ballast at outside of Yantze River Estuary（2012.3.17）—Discharge at outside of Osaka Bay（2012.3.18）				
total abundance（cell/mL）	104.42	35.24	6.02	None
ranges	100.04-111.92	29.21-35.50	5.00-7.13	None

3.4 Chlorophylla

The chlorophyll a (Chl a) concentrations in the three experimental cycles varied between 0.60-1.43 mg/m^3 (Table 3.5). The Chl a concentration decreased 43.4% (first cycle), 26.2% (second cycle), 23.9% (third cycle) in different control tank discharges. The Chl a concentration decreased significantly (> 80%) in the treated tank discharges.
Table3.5 Shipboard testing results of Chl a concentrations in the ballast and discharge waters of Cyeco^{TM} BWMS

Table 3.5 Chla concentration of shipboard testing of Cyeco^{TM} BWMS

First cycle Ballast at Osaka Bay（2011.10.25）— Discharge at Kanmon Strait (2011.10.26）			
Parameters	Influent ballast water (n=3)	discharge water	
		Control tank(n=3)	Treated tank(n=9)
Concentation（mg/m^3）	1.29	0.73	0.09
Range（mg/m^3）	1.14-1.43	0.71-0.75	0.07-0.11
Second cycle Ballast at Kanmon Strait（2011.10.26）— Discharge at outside of Yantze River Estuary（2011.10.27）			
Concentation（mg/m^3）	0.61	0.45	0.09
Range（mg/m^3）	0.60-0.63	0.44-0.45	0.08-0.10

Third cycle Ballast atoutside of Yantze River Estuary（2012.3.17）—Discharge at outside of Osaka Bay（2012.3.18）			
Concentation（mg/m^3）	0.71	0.54	0.08
Range（mg/m^3）	0.38-0.42	0.53-0.55	0.06-0.09

3.5 Heterotrophic bacteria and human pathogens

Table 3.6 listed the testing results of the heterotrophic bacteria and human phthogens in the ballast and discharge waters.

Table 3.6 Shipboard testing results of heterotrophic bacteria and human pathogens in ballast and discharge waters of Cyeco^{TM} -BWMS

First cycle Ballast at Osaka Bay（2011.10.25）— Discharge at Kanmon Strait (2011.10.26）				
Parameters	Ballast water		Discharge water	
	mean(n=3)	range	treated tank（T） mean(n=9)	range
Heterotrophic bacteria（CFU/100mL）	5.6×10^4	（4.4-6.8）$\times10^4$	29.4	18-42
vibrio（CFU/100mL）	2.2×10^3	$(1.8\text{-}2.6)\times10^3$	0	0
vibrio cholerae（CFU/100mL）	/	/	0	0
Escherichia coli（CFU/100mL）	4.1×10^2	$(3.6\text{-}4.6)\times10^2$	22.3	13-32
Intestinal enterococci（CFU/100mL）	45.3	36-48	0	0
Second cycle Ballast at Kanmon Strait（2011.10.26）— Discharge at outside of Yantze River Estuary（2011.10.27）				
Heterotrophic bacteria（CFU/100mL）	2.4×10^4	$(2.2\text{-}2.6)\times10^4$	24.3	18-31
vibrio（CFU/100mL）	1.3×10^3	$(1.1\text{-}1.6)\times10^3$	0	0
vibrio cholerae（CFU/100mL）	/	/	0	0
Escherichia coli（CFU/100mL）	1.7×10^3	$(1.5\text{-}1.9)\times10^3$	7.3	0-36
Intestinal enterococci（CFU/100mL）	1.9×10^2	$(1.8\text{-}2.2)\times10^2$	0	0
Third cycle Ballast at outside of Yantze River Estuary（2012.3.17）—Discharge at outside of Osaka Bay（2012.3.18）				
Heterotrophic bacteria（CFU/100mL）	4.8×10^4	$(3.6\text{-}5.6)\times10^4$	18.3	0-36
vibrio（CFU/100mL）	1.5×10^3	$(1.3\text{-}1.7)\times10^3$	0	0
vibrio cholerae（CFU/100mL）	/	/	0	0
Escherichia coli	83.3	60-100	0	0

（CFU/100mL）				
Intestinal enterococci （CFU/100mL）	0		0	0

The heterotrophic bacteria colonies in all the influent ballast waters were approximately 10^4 CFUs/100mL with relatively lower value at the Strait of Kanmon. The average bacteria colonies in treated tank discharges were 29.4 CFUs/100mL (first cycle), 24.3 CFUs/100mL (second cycle) and 18.3 CFUs/100mL (third cycle), respectively. The conlonies of *vibrio* in the influent ballast waters in three cycles were $>10^3$ CFUs/100mL, but no cultured colonies were detected in the treat tank discharges. The conlonies of *Escherichia coli* varied between 60 CFUs/100mL-1.9×10^3 CFUs/100mL in the influent ballast waters. The average conlonies of *Escherichia coli* in the first and second cycle treated tank discharges was 22.3 CFUs/100mL and 7.3 CFUs/100mL (only cultured in 3 samples). No *Escherichia coli* colonies were detected in the third cycle treated tank discharges. No Intestinal *enterococci* was detected in either ballast or discharge water samples.

4. Evaluation and conclusions

The shipboard testing of treatment efficiency of Cyeco™BWMS manufactured by Shanghai Cyeco Environmental Technology Co.,Ltd was conducted on the cruise ship of Xinjianzhen No.1 from Shanghai-Osaka during October 2010 to March 2012. Following the G8 Guidelines and through 3 experimental cycles, the conclusion was made as follows:

1) The individual density of organisms ≥50 μm varied between 8.69×10^2-3.08×10^4 inds/m^3 in the inflow ballast waters of the three cycles, with an average of 1.61×10^4 inds/m^3， which meet the requirement of G8 Guidelines. The cell density of organisms ≥10 ~50 μm exceeded 10^2 cell/mL in the inflow ballast waters of three cycles, with the highest value in the outside area of Yangtze River Estuary (184.47 cell/mL)and lowest in Kanmon Strait (101.36 cell/mL). The cell density of organisms ≥10 ~50 μm in the control tank discharges was in the range of 30-100 cell/mL, which meet the requirement of G8 Guidelines.

2) No viable organisms ≥50 μm and ≥10 ~50 μm was detected in the treated tank discharges, which meet the requirement of D-2 standard.

3) The average heterotrophic bacteria colonies in the inflow ballast water varied between 2.2×10^4 ~5.6×10^4 CFUs/100mL, all the samples meet the requirement of G8 Guidelines.

4) 4) The average heterotrophic bacteria colonies in the treated tank discharges was 29.4 CFUs/100mL (first cycle), 24.3 CFUs/100mL (second cycle) and 18.3 CFUs/100mL (third cycle). No vibrio cholera and Intestinal enterococci colonies were cultured in the treated ballast waters. The Escherichia coli conolies in the first and second cycle was 22.3 CFUs/100mL and 7.3 CFUs/100mL respectively, and not detected in the third cycle, wich meet the requirement of G8 Guidelines and D-2 Standard.

In summary, the treatment efficiency of Cyeco^{TM} BWMS to the test size organisms all meet the requirement of G8 Guidelines and D-2 Standard.

Table 4.1 Comparison of the test results of Cyeco™ BWMS**with G8 Guidelines and D-2 Standard**

Cycles	**Parameters**	**D-2 Standard and G8 Guideline**		**test results**			**Evaluation**
		Inflow ballast water	**treated discharges**	**Inflow ballast water**	**control discharges**	**treated discharges**	
I	≥50 μm (ind./m^3)	＞100	<10	2.54×10^4	1.17×10^4	no living organism	meet the requirement of D-2 Standard and G8 Guideline
	10-50 μm (cells/mL)	＞100	<10	1.84×10^2	1.12×10^2	no living organism	meet the requirement of D-2 Standard and G8 Guideline
	<10 μm -Bacteria(CFU/100mL)	$\geq10^4$	-	5.6×10^4	/	29.4	meet the requirement of D-2 Standard and G8 Guideline
	Escherichia coli(CFU/100mL)	＞2500	<250	4.1×10^2	/	22.3	meet the requirement of D-2 Standard and G8 Guideline
	Intestinal Enterococci(CFU/100mL)	＞1000	<100	45.3	/	0	meet the requirement of D-2 Standard and G8 Guideline

	Vibrio group	＞10	<1	2.2×10^3	/	0	meet the requirement of D-2 Standard and G8 Guideline
	Vibrio choleerae(CFU/100mL)	＞10	<1	0	/	0	meet the requirement of D-2 Standard and G8 Guideline
II	≥50 μm (ind./m^3)	＞100	<10	2.18×10^4	1.23×10^4	no living organism	meet the requirement of D
	10-50 μm (cell/mL)	＞100	<10	101.36	31.28	no living organism	meet the requirement of D-2 Standard and G8 Guideline
	<10 μm-Bacteria(CFU/100mL)	$\geq10^4$	无规定	2.4×10^4	/	24.3	meet the requirement of D-2 Standard and G8 Guideline
	Escherichia coli(CFU/100mL)	＞2500	<250	1.7×10^3	/	7.3	meet the requirement of D-2 Standard and G8 Guideline
	Intestinal Enterococci(CFU/100mL)	＞1000	<100	1.9×10^2	/	0	meet the requirement of D-2 Standard and G8 Guideline
	Vibrio group(CFU/100mL)	＞10	<1	1.3×10^3	/	0	meet the requirement

							of D-2 Standard and G8 Guideline
	Vibrio choleerae(CFU/100mL)	＞10	<1	0	/	0	meet the requirement of D-2 Standard and G8 Guideline
III	≥50 μm (ind./m^3)	＞100	<10	1.09×10^3	2.15×10^2	no living organism	meet the requirement of D-2 Standard and G8 Guideline
	10-50 μm (cells/mL)	＞100	<10	1.04×10^2	35.24	no living organism	meet the requirement of D-2 Standard and G8 Guideline
	<10 μm -Bacteria(CFU/100mL)	$\geq10^4$	-	4.8×10^4	/	18.3	meet the requirement of D-2 Standard and G8 Guideline
	Escherichia coli(CFU/100mL)	＞2500	<250	83.3	/	0	meet the requirement of D-2 Standard and G8 Guideline
	Intestinal Enterococci(CFU/100mL)	＞1000	<100	0	/	0	meet the requirement of D-2 Standard and G8 Guideline
	Vibrio group(CFU/100mL)	m＞10	<1	1.5×10^3	/	0	meet the requirement

							of D-2 Standard and G8 Guideline
	Vibrio choleerae (CFU/100mL)	＞10	<1	0	/	0	meet the requirement of D-2 Standard and G8 Guideline

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6. Appendix

Appendix 1 The results for environmental parameters of Shipboard Testing of Cyeco^{TM}-BWMS

Test date	Run	Sampling number	Temperature(T°C)	Salinity (PSU)	pH	NTU	TSS(mg/L)	DOC(mg/L)	POC(mg/L)
2011.10.25	Influent water of the 1st test run	S-C1-SP1-B/d	24.6	30.1	7.97	2.98	24.25	1.43	3.39
		S-C1-SP1-M/d	24.2	29.9	8.00	3.68	21.75	1.04	2.36
		S-C1-SP1-E/d	23.9	30.0	7.99	3.86	24.50	1.24	2.77
2011.10.26	Influent water of the 2nd test run	S-C2-SP1-B/d	23.5	34.0	8.13	0.61	2.76	0.55	1.23
		S-C2-SP1-M/d	23.2	34.6	8.14	0.53	3.69	0.71	1.45
		S-C2-SP1-E	23.3	34.4	8.15	0.48	2.57	0.44	1.24
2012.3.17	Influent water of the 3rd test run	S-C3-SP1-B	16.2	32.7	8.03	3.42	5.31	1.23	1.78
		S-C3-SP1-M	14.6	32.5	8.03	1.40	3.54	1.07	1.28
		S-C3-SP1-E	14.1	32.4	8.05	1.45	3.00	0.96	1.12

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Appendix 1 The results for environmental parameters（TRO）of shipboard testing of Cyeco^{TM-}BWMS

Test date	Run	Sampling number	TRO mg/L（as Cl_2）	Average（as Cl_2）	Test date	Run	Sample number	TROmg/L（as Cl_2）	Average（as Cl_2）
2012.3.17	Effluent water of the traetment tank in 3rd test run	S-C3-SP3-B1/d	0.064	0.065	2012.3.17	Effluent water of the traetment tank in 3rd test run	S-C3-SP3-E1/d	0.066	0.066
		S-C3-SP3-B2/d	0.065				S-C3-SP3-E2/d	0.066	
		S-C3-SP3-B3/d	0.065				S-C3-SP3-E3/d	0.065	
		S-C3-SP3-M1/d	0.065	0.065		Effluent water of the control tank in 3rd test run	S-C3-SP4-B/d	0.063	0.062
		S-C3-SP3-M2/d	0.065				S-C3-SP4-M/d	0.062	
		S-C3-SP3-M3/d	0.065				S-C3-SP4-E/d	0.062	

Analyst [illegible] proofreader [illegible]

Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density （ind.·m^{-3}）	Dead density （ind.·m^{-3}）	Total density （ind.·m^{-3}）
2011.10.25	Influent water of the 1st test run	S-C1-SP1-B/a	1	100%	*Labidocera euchaeta*	1	1		28470
				1/200	*Harpacticoida sp.*	11	2200		
				1/200	*Acartia* sp.	7	140		
				1/200	*Paracalanus* sp.	79	15800		
				1/200	*Eucalanus* sp.	1	200		
				100%	*Sagitta* sp.	7	7		
				1/200	Polychaeta larvae	1	200		
				100%	*Lucifer* sp.	1	1		
				1/200	*Corycaeus* sp.	6	120		
				1/200	late Nauplius larvae	49	9800		
				100%	fish egg	1	1		
2011.10.25	Influent water of the 1st test run	S-C1-SP1-M/a	1	1/200	*Paracalanus* sp.	76	15200		30809
				100%	*Eucalanus subcrassus*	2	2		
				1/200	*Acartia* sp.	2	400		
				1/200	*Harpacticoida* sp.	62	12400		
				1/200	Pteropoda	1	200		
				100%	*Sagitta* sp.	2	2		
				1/200	late Nauplius larvae	9	1800		
				1/200	*Oithona* sp.	4	800		
				100%	*Muggiaea* sp.	1	1		
				100%	fish larvae	1	1		
				100%	*Pontellopsis* sp.	1	1		
				100%	Polychaeta larvae	2	2		

Analyst 刘静 Proofreader 王晓伦

Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density (ind.·m^{-3})	Dead density (ind.·m^{-3})	Total density (ind.·m^{-3})
2011.10.25	Influent water of the 1st test run	S-C1-SP1-E/a	1	1/200	*Paracalanus* sp.	42	8400		17007
				1/200	*Harpacticoida* sp.	26	5200		
				1/200	*Oithona* sp.	7	1400		
				100%	*Sagitta* sp.	3	3		
				1/200	*Corycaeus* sp.	1	200		
				1/200	*Acartia* sp.	1	200		
				1/200	late Nauplius larva	8	1600		
				100%	*Pontellopsis* sp.	1	1		
				100%	*Tortanus* sp.	2	2		
				100%	Amphipoda	1	1		
2011.10.26	Effluent water in treated tank of the 1st test run at discharge	S-C1-SP3-B1/a	1	100%	*Oithona* sp.	14		14	34
					Harpacticoida sp.	12		12	
					Paracalanus sp.	4		4	
					Corycaeus sp.	2		2	
					late Nauplius larva	2		2	
2011.10.26	Effluent water in treated tank of the 1st test run at discharge	S-C1-SP3-B2/a	1	100%	*Harpacticoida* sp.	50		50	60
					Oithona sp.	1		1	
					Corycaeus sp.	3		3	
					Paracalanus sp.	4		4	
					Polychaeta larvae	1		1	
					Brachyura zoea	1		1	
2011.10.26	Effluent water in treated tank of the 1st test run at discharge	S-C1-SP3-B3/a	1	100%	*Harpacticoida sp.*	26		26	31
					Acrocalanus sp.	1		1	
					Corycaeus sp.	1		1	
					Oithona sp.	2		2	
					Paracalanus sp.	1		1	

Analyst 谢剑锋 Proofreader 王晓

Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density (ind.$\cdot m^{-3}$)	Dead density (ind.$\cdot m^{-3}$)	Total density (ind.$\cdot m^{-3}$)
2011.10.26	Effluent water in treated tank of the 1st test run at discharge	S-C1-SP3-M1/a	1	100%	*Harpacticoida* sp.	56		56	72
					Paracalanus sp.	9		9	
					Oithona sp.	7		7	
2011.10.26	Effluent water in treated tank of the 1st test run at discharge	S-C1-SP3-M2/a	1	100%	*Harpacticoida* sp.	22		22	27
					Oithona sp.	1		1	
					Corycaeus sp.	2		2	
					Paracalanus sp.	2		2	
2011.10.26	Effluent water in treated tank of the 1st test run at discharge	S-C1-SP3-M3/a	1	100%	*Harpacticoida* sp.	36		36	45
					Paracalanus sp.	8		8	
					Oithona sp.	1		1	
2011.10.26	Effluent water in treated tank of the 1st test run at discharge	S-C1-SP3-E1/a	1	100%	*Harpacticoida* sp.	20		20	34
					Acartia sp.	2		2	
					Ostracoda	1		1	
					Paracalanus sp.	5		5	
					Oithona sp.	6		6	
2011.10.26	Effluent water in treated tank of the 1st test run at discharge	S-C1-SP3-E2/a	1	100%	*Harpacticoida* sp.	32		32	45
					Paracalanus sp.	7		7	
					Corycaeus sp.	2		2	
					Oithona sp.	4		4	
2011.10.26	Effluent water in treated tank of the 1st test run at discharge	S-C1-SP3-E3/a	1	100%	*Oithona* sp.	4		4	31
					Paracalanus sp.	2		2	
					Harpacticoida sp.	25		25	

Analyst 刘革 Proofreader 王晓

Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density (ind.·m^{-3})	Dead density (ind.·m^{-3})	Total density (ind.·m^{-3})
2011.10.26	Effluent water in reference tank of the 1st test run at discharge	S-C1-SP4-B/a	1	1/200	*Paracalanus* sp.	33	6600		12811
				1/200	*Harpacticoida* sp.	28	5600		
				1/200	*Acartia* sp.	1	200		
				1/200	*Oithona* sp.	1	200		
				100%	*Temora* sp.	3	3		
				1/200	*Corycaeus* sp.	1	200		
				100%	*Sagitta* sp.	1	1		
				100%	*Eucalanus subcrassus*	1	1		
				100%	Bivalve larva	6	6		
2011.10.26	Effluent water in reference tank of the 1st test run at discharge	S-C1-SP4-M/a	1	1/200	*Paracalanus* sp.	32	6400		15012
				1/200	*Harpacticoida* sp.	26	5200		
				1/200	*Oithona* sp.	2	400		
				1/200	late Nauplius larvae	14	2800		
				1/200	*Corycaeus* sp.	1	200		
				100%	Brachyura zoea	1	1		
				100%	*Tortanus* sp.	1	1		
				100%	*Centropages furcatus*	3	3		
				100%	Bivalve larva	6	6		
				100%	Nematoda	1	1		
2011.10.26	Effluent water in reference tank of the 1st test run at discharge	S-C1-SP4-E/a	1	1/200	*Paracalanus* sp.	17	3400		7213
				1/200	*Acartia* sp.	1	200		
				100%	*Tortanus* sp.	11	11		
				1/200	*Harpacticoida* sp.	6	1200		
				1/200	late Nauplius larvae	9	1800		
				1/200	*Oithona* sp.	2	400		
				1/200	*Corycaeus* sp.	1	200		
				100%	*Acrocalanus* sp.	2	2		

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Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density (ind.·m^{-3})	Dead density (ind.·m^{-3})	Total density (ind.·m^{-3})
2011.10.26	Influent water of the 2nd test run	S-C2-SP1-B/a	1	1/100	*Paracalanus* sp.	56	5600		22108
				1/100	*Harpacticoida* sp.	21	2100		
				1/100	*Oithona* sp.	14	1400		
				1/100	*Eucalanus subcrassus*	2	200		
				1/100	*Corycaeus* sp.	4	400		
				1/100	Bivalve larvae	9	900		
				1/100	Pteropoda	4	400		
				100%	*Centropages dorsispinat*	1	1		
				1/100	late Nauplius larvae	110	11000		
				100%	*Lucifer* sp.	1	1		
				100%	Brachyura zoea	2	2		
				1/100	*Acartia* sp.	1	100		
				100%	Macrura larvae	4	4		
				1/100	Nematoda	1	100		
2011.10.26	Influent water of the 2nd test run	S-C2-SP1-M/a	1	1/200	*Paracalanus* sp.	49	9800		21011
				1/200	*Corycaeus* sp.	7	1400		
				1/200	*Oithona* sp.	22	4400		
				1/200	Pteropoda	6	1200		
				1/200	late Nauplius larvae	14	2800		
				1/200	Bivalve larvae	2	400		
				1/200	*Acartia* sp.	2	400		
				100%	*Tortanus* sp.	1	1		
				1/200	*Harpacticoida sp.*	3	600		
				100%	Lingula larvae	1	1		
				100%	*Eucalanus* sp.	2	2		
				100%	Brachyura zoea	1	1		
				100%	Macrura larvae	6	6		

Analyst 刘萍 Proofreader 王晓

Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density (ind.·m^{-3})	Dead density (ind.·m^{-3})	Total density (ind.·m^{-3})
2011.10.26	Influent water of the 2nd test run	S-C2-SP1-E/a	1	1/200	*Oithona* sp.	24	4800		22410
				1/200	*Paracalanus* sp.	41	8200		
				1/200	*Corycaeus* sp.	13	2600		
				1/200	*Harpacticoida* sp.	6	1200		
				1/200	Bivalve larvae	1	200		
				1/200	Pteropoda	1	200		
				1/200	late Nauplius larvae	18	3600		
				100%	*Eucalanus subcrassus*	6	6		
				1/200	*Acartia* sp.	2	400		
				100%	Macrura larvae	2	2		
				1/200	*Centropages furcatus*	1	200		
				100%	fish egg	1	1		
				100%	Lingula larvae	1	1		
				1/200	Centropages dorsispinatu	1	200		
				1/200	*Acrocalanus* sp.	2	400		
				1/200	Polychaeta larvae	1	200		
				1/200	*Temora* sp.	1	200		
2011.10.27	Effluent water in treated tank of the 2nd test run at discharge	S-C2-SP3-B1/a	1	100%	*Oithona* sp.	13		13	57
					Paracalanus sp.	4		4	
					Harpacticoida sp.	32		32	
					Corycaeus sp.	2		2	
					Bivalve larvae	2		2	
					Acrocalanus sp.	2		2	
					Acartia sp.	1		1	
					Copepoda larvea	1		1	

Analyst________ Proofreader________

Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density（ind.·m^{-3}）	Dead density（ind.·m^{-3}）	Total density（ind.·m^{-3}）
2011.10.27	Effluent water in treated tank of the 2nd test run at discharge	S-C2-SP3-B2/a	1	100%	*Harpacticoida* sp.	5		5	19
					Corycaeus sp.	2		2	
					Oithona sp.	6		6	
					Noctiluca scintillans	1		1	
					Acartia sp.	1		1	
					Paracalanus sp.	4		4	
2011.10.27	Effluent water in treated tank of the 2nd test run at discharge	S-C2-SP3-B3/a	1	100%	*Harpacticoida* sp.	10		10	33
					Oithona sp.	10		10	
					Paracalanus sp.	5		5	
					Acartia sp.	1		1	
					Acrocalanus sp.	2		2	
					Corycaeus sp.	2		2	
					late Nauplius larvae	1		1	
					Pteropoda	1		1	
					Bivalve larvae	1		1	
2011.10.27	Effluent water in treated tank of the 2nd test run at discharge	S-C2-SP3-M1/a	1	100%	*Harpacticoida* sp.	7		7	13
					Paracalanus sp.	3		3	
					Corycaeus sp.	2		2	
					Oithona sp.	1		1	
2011.10.27	Effluent water in treated tank of the 2nd test run at discharge	S-C2-SP3-M2/a	1	100%	*Oithona* sp.	8		8	37
					Harpacticoida sp.	18		18	
					late Nauplius larvae	3		3	
					Paracalanus sp.	1		1	
					Harpacticoida sp.	7		7	
2011.10.27	Effluent water in treated tank of the 2nd test run at discharge	S-C2-SP3-M3/a	1	100%	*Paracalanus* sp.	3		3	6
					Corycaeus sp.	1		1	
					Oithona sp.	2		2	

Analyst 刘萍 Proofreader 王欣元

Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density (ind.·m^{-3})	Dead density (ind.·m^{-3})	Total density (ind.·m^{-3})
2011.10.27	Effluent water in treated tank of the 2nd test run at discharge	S-C2-SP3-E1/a	1	100%	*Paracalanus* sp.	11		11	32
					Harpacticoida sp.	9		9	
					Oithona sp.	9		9	
					Corycaeus sp.	1		1	
					Temora sp.	1		1	
					Acrocalanus sp.	1		1	
2011.10.27	Effluent water in treated tank of the 2nd test run at discharge	S-C2-SP3-E2/a	1	100%	*Paracalanus* sp.	9		9	24
					Oithona sp.	7		7	
					Harpacticoida sp.	7		7	
					Temora sp.	1		1	
2011.10.27	Effluent water in treated tank of the 2nd test run at discharge	S-C2-SP3-E3/a	1	100%	*Harpacticoida* sp.	7		7	24
					Corycaeus sp.	3		3	
					Oithona sp.	9		9	
					Paracalanus sp.	3		3	
					Lingula larvae	1		1	
					Temora sp.	1		1	
2011.10.27	Effluent water in reference tank of the 2nd test run at discharge	S-C2-SP4-B/a	1	1/100	*Paracalanus* sp.	36	3600		7401
				1/100	*Oithona* sp.	17	1700		
				1/100	*Harpacticoida* sp.	4	400		
				1/100	*Corycaeus* sp.	1	100		
				1/100	*Centropages furcatus*	1	100		
				100%	*Eucalanus subcrassus*	1	1		
				1/100	Bivalve larvae	1	100		
				1/100	Pteropoda	1	100		
				1/100	late Nauplius larvae	12	1200		
				1/100	*Acartia* sp.	1	100		

Analyst 刘萍 Proofreader 王成

Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density（ind.·m^{-3}）	Dead density（ind.·m^{-3}）	Total density（ind.·m^{-3}）
2011.10.27	Effluent water in reference tank of the 2nd test run at discharge	S-C2-SP4-M/a	1	1/200	*Paracalanus* sp.	34	6800		15210
				1/200	*Corycaeus* sp.	4	800		
				1/200	Bivalve larvae	1	200		
				1/200	*Oithona* sp.	16	3200		
				1/200	*Harpacticoida* sp.	2	400		
				1/200	late Nauplius larvae	18	3600		
				100%	*Tortanus* sp.	1	1		
				100%	*Centropages furcatus*	6	6		
				1/200	*Acartia* sp.	1	200		
				100%	*Temora* sp.	1	1		
				100%	Macruralarvae	1	1		
				100%	*Acrocalanus* sp.	1	1		
2011.10.27	Effluent water in reference tank of the 2nd test run at discharge	S-C2-SP4-E/a	1	1/200	*Paracalanus* sp.	21	4200		14209
				1/200	*Harpacticoida* sp.	7	1400		
				1/200	*Corycaeus* sp.	4	800		
				1/200	*Oithona* sp.	15	3000		
				1/200	*Acrocalanus* sp.	1	200		
				1/200	*Acartia* sp.	3	600		
				1/200	late Nauplius larvae	17	3400		
				1/200	*Temora* sp.	1	200		
				1/200	Pteropoda	1	200		
				100%	*Eucalanus* sp.	2	2		
				100%	Bivalve larvae	6	6		
				100%	Macrura larvae	1	1		
				1/200	Polychaeta larvae	1	200		

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Appendix 2 Results for organisums(⩾50 μm) of the Shipboard Testing of Cyceo^{TM}-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density (ind.·m^{-3})	Dead density (ind.·m^{-3})	Total density (ind.·m^{-3})
2012.3.17	Influent water of the 3rd test run	S-C3-SP1B/a	1	1/2	*Paracalanus* sp.	274	548		1328
				1/2	*Harpacticoida* sp.	130	260		
				1/2	Pteropoda	8	16		
				1/2	*Oithona* sp.	69	138		
				1/2	late Nauplius larvae	170	340		
				100%	*Euchaeta* sp.	2	2		
				100%	*Corycaeus* sp.	6	6		
				100%	*Calanus sinicus*		17		
				100%	*Temora turbinata*	1	1		
2012.3.17	Influent water of the 3rd test run	S-C3-SP1M/a	1	1/2	*Paracalanus* sp.		582		1076
				100%	*Oithona similis*	87	87		
				100%	*Corycaeus* sp.	1	2		
				100%	*Harpacticoida* sp.	126	126		
				100%	late Nauplius larvae	260	260		
				100%	*Euchaeta* sp.	1	1		
				100%	*Calanus sinicus*		13		
				100%	Pteropoda	4	4		
				100%	*Sagitta* sp.	1	1		
2012.3.17	Influent water of the 3rd test run	S-C3-SP1E/a	1	100%	*Sagitta* sp.	1	1		865
				100%	*Calanus sinicus*	28	28		
				1/2	*Paracalanus* sp.	268	536		
				100%	*Oithona* sp.	107	107		
				100%	Pteropoda	2	2		
				100%	*Harpacticoida* sp.	140	140		
				100%	late Nauplius larvae	50	50		
				100%	Amphipoda	1	1		

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Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density (ind.·m^{-3})	Dead density (ind.·m^{-3})	Total density (ind.·m^{-3})
2012.3.17	Effluent water in treated tank of the 3rd test run at discharge	S-C3-SP3B1/a	1	100%	*Paracalanus* sp.	8		8	29
					Oithona sp.	3		3	
					Harpacticoida sp.	4		4	
					late Nauplius larva	7		7	
					Corycaeus sp.	3		3	
					Calanus sinicus	2		2	
					Nematoda	1		1	
					Acartia sp.	1		1	
2012.3.17	Effluent water in treated tank of the 3rd test run at discharge	S-C3-SP3B2/a	1	100%	*Oithona* sp.	3		3	14
					Harpacticoida sp.	4		4	
					Paracalanus sp.	6		6	
					Calanus sinicus	1		1	
2012.3.17	Effluent water in treated tank of the 3rd test run at discharge	S-C3-SP3B3/a	1	100%	*Harpacticoida* sp.	2		2	11
					Paracalanus sp.	5		5	
					late Nauplius larvae	2		2	
					Euphausiacea	1		1	
					Corycaeus sp.	1		1	
2012.3.17	Effluent water in treated tank of the 3rd test run at discharge	S-C3-SP3M1/a	1	100%	*Paracalanus* sp.	10		10	23
					Harpacticoida sp.	7		7	
					Oithona sp.	1		1	
					Corycaeus sp.	1		1	
					late Nauplius larvae	2		2	
					Bivalve larva	1		1	
					Acartia sp.	1		1	
2012.3.17	Effluent water in treated tank of the 3rd test run at discharge	S-C3-SP3M2/a	1	100%	late Nauplius larvae	15		15	28
					Acartia sp.	1		1	
					Oithona sp.	2		2	
					Calanus sinicus	1		1	
					Paracalanus sp.	6		6	
					Harpacticoida sp.	3		3	

Analyst 刘萍　　Proofreader 王晓

Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density (ind.·m^{-3})	Dead density (ind.·m^{-3})	Total density (ind.·m^{-3})
2012.3.17	Effluent water in treated tank of the 3rd test run at discharge	S-C3-SP3M3/a	1	100%	*Paracalanus* sp.	4		4	16
					Corycaeus sp.	3		3	
					Bivalve larva	1		1	
					Harpacticoida sp.	7		7	
					Oithona sp.	1		1	
2012.3.17	Effluent water in treated tank of the 3rd test run at discharge	S-C3-SP3E1/a	1	100%	*Harpacticoida* sp.	2		2	6
					Oithona sp.	2		2	
					Paracalanus sp.	1		1	
					Acartia sp.	1		1	
2012.3.17	Effluent water in treated tank of the 3rd test run at discharge	S-C3-SP3E2/a	1	100%	*Oithona* sp.	4		4	21
					Bivalve larva	1		1	
					Paracalanus sp.	10		10	
					Harpacticoida sp.	2		2	
					Corycaeus sp.	4		4	
2012.3.17	Effluent water in treated tank of the 3rd test run at discharge	S-C3-SP3E3/a	1	100%	*Corycaeus* sp.	4		4	24
					Calanus sinicus	1		1	
					Paracalanus sp.	16		16	
					Oithona sp.	3		3	
2012.3.18	Effluent water in reference tank of the 2nd test run at discharge	S-C3-SP4B/a	1	100%	*Calanus sinicus*	4	4		235
					Corycaeus sp.	2	2		
					Euchaeta sp.	2	2		
					Harpacticoida sp.	57	57		
					Paracalanus sp.	126	126		
					Oithona sp.	43	43		
					Chaetognatha	1	1		

Analyst 刘潇 Proofreader 王晓

Appendix 2 Results for organisums(≥50 μm) of the Shipboard Testing of Cyceo™-BWMS

Sampling date	Run	Sample number	Filter volume(m^3)	count proportion of concentration volume	Latin name	number of counting volume	Aalive density (ind.·m^{-3})	Dead density (ind.·m^{-3})	Total density (ind.·m^{-3})
2012.3.18	Effluent water in reference tank of the 2nd test run at discharge	S-C3-SP4M/a	1	100%	*Calanus sinicus*	2	2		
					Pteropoda	1	1		79
					Paracalanus sp.		45		
					Nematoda		3		
					Harpacticoida sp.		12		
					Oithona sp.		14		
					late Nauplius larvae		2		
2012.3.18	Effluent water in reference tank of the 2nd test run at discharge	S-C3-SP4E/a	1	100%	*Oithona* sp.		108		
					Harpacticoida sp.		42		332
					Paracalanus sp.		136		
					Calanus sinicus		9		
					late Nauplius larvae		32		
					Euchaeta sp.	1	1		
					Labidocera sp.	1	1		
					Pteropoda	3	3		

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco™-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species: Phyta	Dominant Species: Latin	Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
2011.10.25	Influent water of the 1st test run	S-C1-SP1-B/b	1	50	Diatoms	*Actinoptychus* sp.	23	1.92		200.67
						Rhizosolenia stolterforthii	30	2.50		
						Thalassionema nitzschioides	390	32.50		
						Thalassiosira sp.	39	3.25		
						Nitzschia sp.	21	1.75		
						Skeletonema costatum	339	28.25		
						Coscinodiscus sp.	93	7.75		
						Rhizosolenia styliformis	21	1.75		
						Coscinodiscus oculus-iridis	9	0.75		
						Bellerochea malleus	246	20.50		
						Podocystis spathulata	42	3.50		
					Dinoflgellate	*Peridinium depressum*	9	0.75		
						Gymnodinium sp.	21	1.75		
						Gyrodinium spp.	21	1.75		
					Chrysophyta	*Dictyocha fibula*	33	2.75		
						Phaeocystis sp.	669	55.75		
						Ebria tripartita	51	4.25		
					Others		351	29.25		
2011.10.25	Influent water of the 1st test run	S-C1-SP1-M/b	1	50	Diatoms	*Thalassiothrix frauenfeldii*	237	19.75		
						Ditylum brightwelli	9	0.75		
						Actinoptychus sp.	9	0.75		
						Rhizosolenia stolterforthii	39	3.25		
						Chaetoceros sp.	834	69.50		
						Biddulphia longicruris	9	0.75		
						Thalassiosira sp.	39	3.25		
						Nitzschia sp.	72	6.00		
						Bacteriastrum sp	114	9.50		
						Rhizosolenia styliformis	72	6.00		
						Rhizosolenia alata f. *indica*	9	0.75		

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco™-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species: Phyta	Dominant Species: Latin	Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
2011.10.25	Influent water of the 1st test run	S-C1-SP1-M/b	1	50	Dinoflgellate	*Ceratium lineatum*	9	0.75		180.50
						Scrippsiella spp	9	0.75		
						Gymnodinium sp	30	2.50		
					Chrysophyta	*Dictyocha fibula*	21	1.75		
						Phaeocystis sp	102	8.50		
					Others		552	46.00		
2011.10.25	Influent water of the 1st test run	S-C1-SP1-E/b	1	50	Diatoms	*Diploneis* sp.	12	1.00		172.25
						Actinoptychus sp.	27	2.25		
						Paralia sulcata	69	5.75		
						Thalassionema nitzschioides	687	57.25		
						Nitzschia sp.	6	0.50		
						Skeletonema costatum	63	5.25		
						Actinocyclus sp.	21	1.75		
						Coscinodiscus sp.	33	2.75		
						Coscinodiscus centralis	6	0.50		
						Coscinodiscus spinosus	6	0.50		
						Bellerochea malleus	213	17.75		
						Podocystis spathulata	54	4.50		
					Dinoflgellate	*Ceratium fusus*	6	0.50		
					Chrysophyta	*Phaeocystis* sp.	378	31.50		
						Distephanus speculum	6	0.50		
						Dictyocha fibula	54	4.50		
						Ebria tripartita	42	3.50		
					Others	Others	384	32.00		

Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco™-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species Pyhta	Dominant Species Latin	Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
2011.10.26	deballast water of the 1st test run	S-C1-SP3-B1/b	1	15	Diatoms	*Diploneis* sp.	18		0.450	6.38
						Actinoptychus sp.	3		0.075	
						Rhizosolenia stolterforthii	6		0.150	
						Chaetoceros sp.	18		0.450	
						Thalassionema nitzschioides	18		0.450	
						Thalassiosira sp.	33		0.825	
						Nitzschia sp.	9		0.225	
						Skeletonema costatum	24		0.600	
						Coscinodiscus sp.	18		0.450	
						Chaetoceros curvisetus	57		1.425	
						Podocystis spathulata	9		0.225	
					Others		42		1.050	
		S-C1-SP3-B2/b	1	15	Diatoms	*Thalassiothrix frauenfeldii*	33		0.825	6.48
						Actinoptychus sp.	5		0.125	
						Pleurosigma sp.	9		0.225	
						Thalassionema nitzschioides	45		1.125	
						Thalassiosira sp.	9		0.225	
						Coscinodiscus sp.	20		0.500	
						Rhizosolenia delicatula	9		0.225	
						Podocystis spathulata	6		0.150	
					Chrysophyta	*Dictyocha fibula*	9		0.225	
					Others		114		2.850	
		S-C1-SP3-B3/b	1	15	Diatoms	*Ditylum brightwelli*	9		0.225	7.55
						Thalassionema nitzschioides	93		2.325	
						Thalassiosira sp.	9		0.225	
						Nitzschia sp.	9		0.225	
						Coscinodiscus sp.	32		0.800	
						Bellerochea malleus	33		0.825	
					Dinoflgellate	*Gymnodinium* sp.	9		0.225	
					Chrysophyta	*Dictyocha fibula*	33		0.825	
					Others		75		1.875	

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco^{TM}-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species: Phyta	Dominant Species: Latin	Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
2011.10.26	deballast water of the 1st test run	S-C1-SP3-M1/b	1	15	Diatoms	*Thalassiothrix frauenfeldii*	36		0.900	7.90
						Pleurosigma sp.	3		0.075	
						Melosira sulcata	54		1.350	
						Nitzschia sp.	6		0.150	
						Skeletonema costatum	60		1.500	
						Coscinodiscus sp.	26		0.650	
						Rhizosolenia styliformis	6		0.150	
						Podocystis spathulata	18		0.450	
					Dinoflgellate	*Ceratium fusus*	2		0.050	
					Chrysophyta	*Dictyocha fibula*	4		0.100	
					Others		101		2.525	
		S-C1-SP3-M2/b	1	15	Diatoms	*Actinoptychus* sp.	9		0.225	5.18
						Pleurosigma sp.	3		0.075	
						Biddulphia longicruris	3		0.075	
						Thalassionema nitzschioides	18		0.450	
						Thalassiosira sp.	9		0.225	
						Coscinodiscus sp.	18		0.450	
						Rhizosolenia styliformis	6		0.150	
						Naviculamembranacea	18		0.450	
						Bellerochea malleus	54		1.350	
						Podocystis spathulata	9		0.225	
					Chrysophyta	*Dictyocha fibula*	9		0.225	
					Others		51		1.275	
					Diatoms	*Actinoptychus* sp.	3		0.075	6.35
						Chaetoceros sp.	19		0.475	
						Biddulphia longicruris	6		0.150	
						Coscinodiscus sp.	18		0.450	
						Bellerochea malleus	33		0.825	
						Podocystisspathulata	9		0.225	
						Ebria tripartita	9		0.225	
					Chrysophyta	*Dictyocha fibula*	33		0.825	
					Others		124		3.100	

Analyst 李超 Proofreader 孙[illegible]

Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco™-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Pyhta	Latin				
2011.10.26	deballast water of the 1st test run	S-C1-SP3-E1/b	1	15	Diatoms	*Thalassiothrix frauenfeldii*	12		0.300	5.33
						Diploneis sp.	6		0.150	
						Ditylum brightwelli	6		0.150	
						Pleurosigma sp..	3		0.075	
						Thalassionema nitzschioides	6		0.150	
						Coscinodiscus sp.	33		0.825	
						Rhizosolenia styliformis	12		0.300	
						Podocystis spathulata	12		0.300	
					Chrysophyta	*Dictyocha fibula*	33		0.825	
					Others		90		2.250	
		S-C1-SP3-E2/b	1	15	Diatoms	*Actinoptychus* sp.	9		0.225	7.83
						Pleurosigma sp.	3		0.075	
						Melosira sulcata	23		0.575	
						Rhizosolenia stolterforthii	18		0.450	
						Thalassionema nitzschioides	66		1.650	
						Thalassiosira sp.	14		0.350	
						Nitzschia sp.	9		0.225	
						Coscinodiscus sp.	18		0.450	
						Rhizosolenia styliformis	9		0.225	
						Navicula membranacea	18		0.450	
					Chrysophyta	*Dictyocha fibula*	27		0.675	
					Others		99		2.475	
		S-C1-SP3-E3/b	1	15	Diatoms	*Diploneis* sp.	9		0.225	3.13
						Rhizosolenia stolterforthii	9		0.225	
						Thalassiosira sp.	18		0.450	
						Coscinodiscus sp.	9		0.225	
					Chrysophyta	*Dictyocha fibula*	9		0.225	
					Others		71		1.775	

Analyst

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco^{TM}-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Pyhta	Latin				
2011.10.26	deballast water of the 1st test run	S-C1-SP4-B/b	1	50	Diatoms	*Thalassiothrix frauenfeldii*	8	0.63		122.29
						Diploneis sp.	8	0.63		
						Biddulphia aurita	28	2.29		
						Pleurosigma sp.	8	0.63		
						Melosira sulcata	138	11.46		
						Chaetoceros sp.	70	5.83		
						Biddulphia longicruris	8	0.63		
						Thalassionema nitzschioides	140	11.67		
						Thalassiosira sp.	15	1.25		
						Nitzschia sp.	8	0.63		
						Skeletonema costatum	80	6.67		
						Coscinodiscus sp.	95	7.92		
						Cyclotella sp.	8	0.63		
						Rhizosolenia styliformis	8	0.63		
						Nitzschia paradoxa	75	6.25		
						Chaetoceros lorenzianus	20	1.67		
						Bacteriastrum hyalinum	8	0.63		
						Bellerochea malleus	218	18.13		
						Podocystis spathulata	43	3.54		
					Dinoflgellate	*Gymnodinium* sp.	8	0.63		
						Gyroirale sp.	8	0.63		
					Chrysophyta	*Phaeocystis sp.*	228	18.96		
						Dictyocha fibula	28	2.29		
						Ebria tripartita	28	2.29		
					Others		190	15.83		
2011.10.26	contrasted deballast water of the 1st test run	S-C1-SP4-M/b	1	50	Diatoms	*Thalassiothrix frauenfeldii*	26	2.17		
						Actinoptychus sp.	8	0.63		
						Pleurosigma sp.	15	1.25		
						Melosira sulcata	70	5.83		
						Rhizosolenia stolterforthii	8	0.63		
						Chaetoceros sp.	83	6.88		

Analyst 李超 Proofreader 孙军

Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco^{TM}-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Pyhta	Latin				
2011.10.26	contrasted deballast water of the 1st test run	S-C1-SP4-M/b	1	50	Diatoms	*Thalassionema nitzschioides*	318	26.46		98.96
						Nitzschia sp.	44	3.67		
						Skeletonema costatum	20	1.67		
						Coscinodiscus sp.	65	5.42		
						Synedra sp.	8	0.63		
						Rhizosolenia styliformis	20	1.67		
						Bellerochea malleus	15	1.25		
						Podocystis spathulata	43	3.54		
					Chrysophyta	*Dictyocha fibula*	28	2.29		
						Phaeocystis sp.	213	17.71		
					Cyanophyta	*Trichodesmium* sp.	208	17.29		
2011.10.26	contrasted deballast water of the 1st test run	S-C1-SP4-E/b	1	50	Diatoms	*Thalassiothrix frauenfeldii*	43	3.54		114.38
						Diploneis sp.	20	1.67		
						Ditylum brightwelli	15	1.25		
						Rhizosolenia stolterforthii	8	0.63		
						Chaetoceros sp.	43	3.54		
						Thalassionema nitzschioides	263	21.88		
						Nitzschia sp.	43	3.54		
						Skeletonema costatum	75	6.25		
						Actinocyclus sp.	15	1.25		
						Coscinodiscus sp.	28	2.29		
						Guinardia flaccida	20	1.67		
						Eucampia zoodiacus	8	0.63		
						Bellerochea malleus	295	24.58		
						Ebria tripartita	15	1.25		
						Podocystis spathulata	8	0.63		
					Dinoflgellate	*Gyrodinium spirale*	8	0.63		
					Chrysophyta	*Dictyocha fibula*	35	2.92		
						Phaeocystis sp.	70	5.83		
					Others		365	30.42		

Analyst 李利达　　Proofreader 孙库

Appendix 3 Results for organisms (10-50μm) of the shipboard Testing of Cyeco™-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Phyta	Latin				
2011.10.26	Influent water of the 2nd test run	S-C2-SP1-B/b	1	50	Diatoms	*Pinnularia* sp.	12	1.00		97.92
						Thalassiothrix frauenfeldii	56	4.67		
						Diploneis sp.	44	3.67		
						Ditylum brightwelli	12	1.00		
						Actinoptychus sp.	32	2.67		
						Coscinodiscus spp.	131	10.92		
						Biddulphia aurita	68	5.67		
						Pleurosigma sp.	12	1.00		
						Paralia sulcata	32	2.67		
						Chaetoceros sp.	68	5.67		
						Biddulphia longicruris	12	1.00		
						Bellerochea malleus	68	5.67		
					Chrysophyta	*Dictyocha fibula*	12	1.00		
						Phaeocystis sp.	220	18.33		
					Cyanophyta	*Trichodesmium* sp.	180	15.00		
					Others		216	18.00		
2011.10.26	Influent water of the 2nd test run	S-C2-SP1-M/b	1	50	Diatoms	*Thalassiothrix frauenfeldii*	96	8.00		107.50
						Diploneis sp.	56	4.67		
						Actinoptychus sp.	12	1.00		
						Coscinodiscus spp.	90	7.50		
						Pleurosigma sp.	24	2.00		
						Bellerochea malleus	96	8.00		
						Paralia sulcata	124	10.33		
						Biddulphia longicruris	32	2.67		
						Thalassionema nitzschioides	32	2.67		
						Thalassiosira sp.	32	2.67		
						Skeletonema costatum	32	2.67		
						Actinocyclus sp.	12	1.00		
					Dinoflgellate	*Ceratium lineatum*	24	2.00		
					Chrysophyta	*Dictyocha fibula*	24	2.00		
						Phaeocystis sp.	188	15.67		
					Cyanophyta	*Trichodesmium* sp.	212	17.67		
					Others		204	17.00		

Analyst 李某某 Proofreader 孙某

Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco™-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Phyta	Latin				
2011.10.26	Influent water of the 2nd test run	S-C2-SP1-E/b	1	50	Diatoms	*Pinnularia* sp.	24	2.00		98.67
						Thalassiothrix frauenfeldii	76	6.33		
						Diploneis sp.	24	2.00		
						Actinoptychus sp.	24	2.00		
						Biddulphia aurita	12	1.00		
						Pleurosigma sp.	24	2.00		
						Coscinodiscus spp.	88	7.33		
						Rhizosolenia stolterforthii	12	1.00		
						Chaetoceros sp.	44	3.67		
						Biddulphia longicruris	12	1.00		
						Nitzschia sp.	84	7.00		
						Cyclotella sp.	24	2.00		
						Synedra sp.	12	1.00		
						Navicula membranacea	68	5.67		
						Bellerochea malleus	84	7.00		
						Thalassionema nitzschioides	52	4.33		
						Navicula sp .	12	1.00		
					Dinoflgellate	*Ceratium lineatum*	12	1.00		
					Chrysophyta	*Dictyocha fibula*	12	1.00		
						Phaeocystis sp.	208	17.33		
						Distephanus speculum	12	1.00		
					Others		264	22.00		
2011.10.27	deballast water of the 2nd test run	S-C2-SP3-B1/b	1	28	Diatoms	*Diploneis* sp.	6		0.28	4.25
						Paralia sulcata	19		0.89	
						Chaetoceros sp.	6		0.28	
						Thalassiosira sp.	3		0.14	
						Biddulphia reticulate	6		0.28	
						Guinardia flaccida	3		0.14	
						Bacteriastrum sp.	3		0.14	
					Dinoflgellate	*Prorocentrum* sp.	3		0.14	
						Gymnodinium sp.	6		0.28	
					Others		36		1.68	

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco™-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Phyta	Latin				
2011.10.27	deballast water of the 2nd test run	S-C2-SP3-B2/b	1	12	Diatoms	*Thalassiothrix frauenfeldii*	28		0.56	4.44
						Diploneis sp.	18		0.36	
						Actinoptychus sp.	6		0.12	
						Rhizosolenia stolterforthii	6		0.12	
						Chaetoceros sp.	22		0.44	
						Nitzschia sp.	34		0.68	
						Biddulphia reticulate	6		0.12	
						Rhizosolenia styliformis	6		0.12	
					Chrysophyta	*Dictyocha fibula*	12		0.24	
					Others		84		1.68	
2011.10.27	deballast water of the 2nd test run	S-C2-SP3-B3/b	1	12	Diatoms	*Thalassiothrix frauenfeldii*	8		0.16	3.24
						Diploneis sp.	4		0.08	
						Paralia sulcata	38		0.76	
						Thalassiosira sp.	12		0.24	
						Nitzschia sp.	12		0.24	
						Cyclotella sp.	8		0.16	
					Chrysophyta	*Dictyocha fibula*	8		0.16	
					Dinoflgellate	*Gymnodinium* sp.	10		0.20	
						Protoperidinium bipes	4		0.08	
						Dinophysis sp.	4		0.08	
					Others		54		1.08	
2011.10.27	deballast water of the 2nd test run	S-C2-SP3-M1/b	1	20	Diatoms	*Thalassiothrix frauenfeldii*	10		0.33	3.47
						Rhizosolenia stolterforthii	4		0.13	
						Chaetoceros sp.	10		0.33	
						Thalassiosira sp.	4		0.13	
						Nitzschia sp.	14		0.47	
						Rhizosolenia styliformis	10		0.33	
					Chrysophyta	*Dictyocha fibula*	10		0.33	
						Distephanus speculum	4		0.13	
					Others		38		1.27	

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco^{TM}-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Phyta	Latin				
2011.10.27	deballast water of the 2nd test run	S-C2-SP3-M2/b	1	8	Diatoms	*Thalassiothrix frauenfeldii*	22		1.83	3.09
						Pleurosigma sp.	6		0.08	
						Biddulphia longicruris	2		0.03	
						Thalassiosira sp.	6		0.08	
						Nitzschia sp.	6		0.08	
						Coscinodiscus sp.	4		0.05	
						Cyclotella sp.	6		0.08	
					Others		64		0.85	
2011.10.27	deballast water of the 2nd test run	S-C2-SP3-M3/b	1	16	Diatoms	*Thalassiothrix frauenfeldii*	26		0.69	3.57
						Pleurosigma sp.	6		0.16	
						Biddulphia longicruris	16		0.43	
						Nitzschia sp.	6		0.16	
						Skeletonema costatum	34		0.91	
						Cyclotella sp.	6		0.16	
					Chrysophyta	*Dictyocha fibula*	6		0.16	
					Others		34		0.91	
2011.10.27	deballast water of the 2nd test run	S-C2-SP3-E1/b	1	16	Diatoms	*Thalassiothrix frauenfeldii*	12		0.32	3.25
						Pleurosigma sp.	6		0.16	
						Thalassiosira sp.	6		0.16	
						Nitzschia sp.	12		0.32	
						Coscinodiscu s sp.	6		0.16	
						Guinardia flaccida	28		0.75	
					Chrysophyta	*Dictyocha fibula*	6		0.16	
					Others		46		1.23	
	deballast water of the 2nd test run	S-C2-SP3-E2/b	1	18	Diatoms	*Thalassiothrix frauenfeldii*	16		0.48	4.08
						Actinoptychu s sp.	6		0.18	
						Paralia sulcata	16		0.48	
						Biddulphia longicruris	12		0.36	
						Cyclotella sp.	6		0.18	
						Chaetoceros laevis	26		0.78	
					Chrysophyta	*Dictyocha fibula*	6		0.18	
					Others		48		1.44	

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco^{TM}-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Phyta	Latin				
2011.10.27	deballast water of the 2nd test run	S-C2-SP3-E3/b	1	10	Diatoms	*Thalassiothrix frauenfeldii*	16		0.27	2.15
						Diploneis sp.	3		0.05	
						Actinoptychus sp.	3		0.05	
						Pleurosigma sp.	3		0.05	
						Paralia sulcata	14		0.23	
						Rhizosolenia stolterforthii	3		0.05	
						Biddulphia longicruris	6		0.10	
						Nitzschia sp.	3		0.05	
						Cyclotella sp.	3		0.05	
						Synedra sp.	3		0.05	
						Rhizosolenia styliformis	6		0.10	
					Chrysophyta	*Dictyocha fibula*	11		0.18	
					Others		55		0.92	
2011.10.27	contrasted deballast water of the 2nd test run	S-C2-SP4-B/b	1	50	Diatoms	*Pinnulari* a sp.	12	1.00		34.17
						Thalassiothrix frauenfeldii	38	3.17		
						Diploneis sp.	3	0.25		
						Ditylum brightwelli	6	0.50		
						Actinoptychus sp.	13	1.08		
						Pleurosigma sp.	6	0.50		
						Biddulphia longicruris	16	1.33		
						Thalassiosira sp.	36	3.00		
						Nitzschia sp.	12	1.00		
						Coscinodiscus sp.	29	2.42		
						Ceratium lineatum	7	0.58		
						Guinardia flaccida	6	0.50		
						Biddulphia sp.	12	1.00		
						Coscinodiscus oculus-iridis	6	0.50		
						Bellerochea malleus	106	8.83		
						Ebria tripartita.	12	1.00		
					Others		90	7.5		

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco^{TM}-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Phyta	Latin				
2011.10.27	contrasted deballast water of the 2nd test run	S-C2-SP4-M/b	1	50	Diatoms	*Thalassiothrix frauenfeldii*	16	1.33		29.50
						Diploneis sp.	8	0.67		
						Ditylum brightwelli	6	0.50		
						Actinoptychus sp.	8	0.67		
						Pleurosigma sp.	6	0.50		
						Chaetoceros sp.	22	1.83		
						Thalassionema nitzschioides	38	3.17		
						Nitzschia sp.	10	0.83		
						Coscinodiscus sp.	12	1.00		
						Guinardia flaccida	12	1.00		
						Coscinodiscus oculus-iridis	4	0.33		
						Bellerochea malleus	122	10.17		
						Ebria tripartita	6	0.50		
					Others		84	7.00		
2011.10.27	contrasted deballast water of the 2nd test run	S-C2-SP4-E/b	1	50	Diatoms	*Thalassiothrix frauenfeldii*	10	0.83		30.17
						Actinoptychus sp.	6	0.50		
						Pleurosigma sp.	6	0.50		
						Thalassiosira sp.	16	1.33		
						Nitzschia sp.	6	0.50		
						Coscinodiscus sp.	4	0.33		
						Rhizosolenia styliformis	6	0.50		
						Navicula sp.	10	0.83		
						Bellerochea malleus	138	11.50		
					Chrysophyta	*Dictyocha fibula*	6	0.50		
						Phaeocystis sp	64	5.33		
					Cyanophyta	*Trichodesmium* sp.	56	4.67		
					Others		34	2.83		

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco^{TM}-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Phyta	Latin				
2012.3.18	Influent water of the 3rd test run	S-C3-SP1-B/b	1	50	Diatoms	*Skeletonema costatum*	154	12.83		101.29
						Thalassiosira sp.	121	10.08		
						Chaetoceros curvisetus	176	14.67		
						Chaetoceros sp.	61	5.04		
						Nitzschia sp.	44	3.67		
						Eucampia zoodiacus	187	15.58		
						Pseudo-nitzschia pungens	259	21.54		
						Coscinodiscus spp.	39	3.21		
					Dinoflgellate	Dinoflagellates	116	9.63		
					Others		61	5.04		
		S-C3-SP1-M/b	1	50	Diatoms	*Thalassiosira* sp	39	3.21		111.92
						Eucampia zoodiacus	160	13.29		
						Chaetoceros sp.	99	8.25		
						Rhizosolenia setigera	33	2.75		
						Skeletonema costatum	231	19.25		
						Pseudo-nitzschia pungens	282	23.46		
						Chaetoceros curvisetus	154	12.83		
						Nitzschia sp.	116	9.63		
					Dinoflgellate	Dinoflagellates	160	13.29		
					Others		72	5.96		
		S-C3-SP1-E/b	1	50	Diatoms	*Eucampia zoodiacus*	182	15.13		100.04
						Chaetoceros curvisetus	242	20.17		
						Nitzschia sp.	39	3.21		
						Paralia sulcata	88	7.33		
						Coscinodiscus	39	3.21		
						Thalassiosira sp.	138	11.46		
						Pseudo-nitzschia pungens	209	17.42		
						Pleurosigma sp.	6	0.46		
					Dinoflgellate	Dinoflagellata	174	14.50		
					Others		86	7.17		

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco™-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Phyta	Latin				
2012.3.19	deballast water of the 3rd test run	S-C3-SP3-B1/b	1	15	Diatoms	*Nitzschia* sp.	75		1.88	5.88
						Pseudo-nitzschia pungens	110		2.75	
						Coscinodiscus sp.	50		1.25	
		S-C3-SP3-B2/b	1	15	Diatoms	*Coscinodiscus* sp.	105		2.63	7.13
						Chaetoceros castracanei	30		0.75	
						Pseudo-nitzschia pungens	140		3.50	
						Pleurosigma sp.	10		0.25	
		S-C3-SP3-B3/b	1	15	Diatoms	*Chaetoceros* sp.	90		2.25	5.75
						Pseudo-nitzschia pungens	105		2.63	
						Coscinodiscus sp.	35		0.88	
2012.3.19	deballast water of the 3rd test run	S-C3-SP3-M1/b	1	15	Diatoms	*Dinoflagellata* spp.	80		2.00	6.50
						Nitzschia sp.	20		0.50	
						Pseudo-nitzschia pungens	140		3.50	
						Cyclotella sp.	20		0.50	
		S-C3-SP3-M2/b	1	15	Diatoms	*Dinoflagellata* spp.	40		1.00	5.00
						Cyclotella sp.	10		0.25	
						Chaetoceros sp.	60		1.50	
						Pseudo-nitzschia pungens	90		2.25	
		S-C3-SP3-M3/b	1	15	Diatoms	*Dinoflagellata* spp	40		1.00	5.25
						Thalassiosira sp	20		0.50	
						Coscinodiscus	40		1.00	
						Pseudo-nitzschia pungens	110		2.75	
2012.3.19	deballast water of the 3rd test run	S-C3-SP3-E1/b	1	15	Diatoms	*Thalassiosira* sp.	25		0.63	5.63
						Eucampia zoodiacus	60		1.50	
						Coscinodiscus sp.	30		0.75	
						Pseudo-nitzschia pungens	75		1.88	
					Dinoflgellate	Dinoflagellata spp.	35		0.88	

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Appendix 3 Results for organisms（10-50μm）of the shipboard Testing of Cyeco™-BWMS

Sampling date	Testing run	Sample number	Volume of filtering (L)	concentrated volume (mL)	Dominant Species		Count	Alive density (cells/mL)	Dead density (cells/mL)	Total density (cells/mL)
					Phyta	Latin				
2012.3.19	deballast water of the 3rd test run	S-C3-SP3-E2/b	1	15	Diatoms	*Thalassiosira* sp.	70		1.75	6.13
						Chaetoceros sp.	40		1.00	
						Eucampia zoodiacus	35		0.88	
						Coscinodiscus	15		0.38	
						Pseudo-nitzschia pungens	70		1.75	
					Chrysophyta	*Dictyocha fibula*	10		0.25	
					Dinoflgellate	*Ceratium lineatum*	5		0.13	
2012.3.19	deballast water of the 3rd test run	S-C3-SP3-E3/b	1	15	Diatoms	*Thalassiosira* sp.	35		0.88	6.88
						Chaetoceros spp.	100		2.50	
						Eucampia zoodiacus	45		1.13	
						Coscinodiscus sp.	30		0.75	
						Pseudo-nitzschia pungens	45		1.13	
					Chrysophyta	*Dictyocha fibula*	20		0.50	
2012.3.19	contrasted deballast water of the 3rd test run	S-C3-SP4-B/b	1	55	Diatoms	*Coscinodiscus*	50		4.62	29.21
						Eucampia zoodiacus	113	10.40		
						Chaetoceros spp.	67	6.11		
						Rhizosolenia sp.	25	2.31		
						Rhizosolenia setigera	13	1.16		
						Pseudo-nitzschia pungens	50	4.62		
		S-C3-SP4-M/b	1	50	Diatoms	*Dinoflagellates*	63	5.25		41.00
						Thalassiosira sp.	42	3.50		
						Chaetoceros curvisetus	84	7.00		
						Nitzschia sp.	21	1.75		
						Eucampia zoodiacus	81	6.75		
						Chaetoceros spp.	96	8.00		
						Rhizosolenia setigera	21	1.75		
						Chaetoceros paradoxus	84	7.00		
		S-C3-SP4-E/b	1	50	Diatoms	*Thalassiosira* sp.	63	5.25		35.50
						Nitzschia sp.	21	1.75		
						Chaetoceros spp.	129	10.75		
						Chaetoceros paradoxus	66	5.50		
						Eucampia zoodiacus	147	12.25		

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Appendix 4 Results for chlorophyll-a of the Shipboard Testing of Cyeco™-BWMS

Sampling data	Testing run	Sample number	Volume of filtering (cm^3)	Fluorescence values before acidification (Rb)	Fluorescence values after acidification (Rb)	concertraation of chlorophyll-a ($mg \cdot m^{-3}$)	Mean ($mg \cdot m^{-3}$)
2011.10.25	Influent water of control tank of the 1st test run	S-C1-SP1-B	250	35.2	20.1	1.14	1.29
		S-C1-SP1-M	200	32.8	19.0	1.31	
		S-C1-SP1-E	200	36.0	20.9	1.43	
2011.10.26	Deballast water of treated tank of the 1st test run	S-C1-SP3-B1	250	4.1	3.1	0.08	0.08
		S-C1-SP3-B2	250	4.5	3.6	0.07	
		S-C1-SP3-B3	250	4.4	3.5	0.09	
		S-C1-SP3-M1	250	4.0	3.1	0.07	0.09
		S-C1-SP3-M2	250	4.7	3.7	0.08	
		S-C1-SP3-M3	250	3.4	2.2	0.11	
		S-C1-SP3-E1	250	4.7	3.5	0.09	0.09
		S-C1-SP3-E2	250	4.6	3.5	0.10	
		S-C1-SP3-E3	250	3.8	3.0	0.08	
2011.10.26	deballast water of control tank of the 1st test run	S-C1-SP4-E	250	19.9	12.0	0.75	0.73
		S-C1-SP4-M	250	19.4	11.6	0.74	
		S-C1-SP4-E	250	20.1	12.6	0.71	
2011.10.26	Influent water of control tank of the 2nd test run	S-C2-SP1-B	250	17.9	9.8	0.61	0.61
		S-C2-SP1-M	250	17.5	9.6	0.60	
		S-C2-SP1-E	250	18.3	10.0	0.63	
2011.10.26	Deballast water of treated tank of the 2nd test run	S-C2-SP3-B1	250	4.2	2.9	0.10	0.09
		S-C2-SP3-B2	250	3.8	2.8	0.08	
		S-C2-SP3-B3	250	4.1	2.8	0.10	
		S-C2-SP3-M1	250	4.8	3.6	0.09	0.09
		S-C2-SP3-M2	250	3.9	2.8	0.08	
		S-C2-SP3-M3	250	4.4	3.1	0.10	
		S-C2-SP3-E1	250	4.5	3.2	0.10	0.09
		S-C2-SP3-E2	250	4.2	3.0	0.09	
		S-C2-SP3-E3	250	4.8	3.7	0.08	

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Appendix 4 Results for chlorophyll-a of the Shipboard Testing of Cyeco™-BWMS

Sampling data	Testing run	Sample number	Volume of filtering (cm^3)	Fluorescence values before acidification (Rb)	Fluorescence values after acidification (Rb)	concertraation of chlorophyll-a ($mg \cdot m^{-3}$)	Mean ($mg \cdot m^{-3}$)
2011.10.27	deballast water of control tank of the 2nd test run	S-C2-SP4-B	250	14.4	8.6	0.44	0.45
		S-C2-SP4-M	250	15.0	9.0	0.45	
		S-C2-SP4-E	250	14.7	8.8	0.45	
2012.3.17	Influent water of control tank of the 3rd test run	S-C3-SP1-B	250	18.6	9.3	0.70	0.71
		S-C3-SP1-M	250	18.8	9.4	0.71	
		S-C3-SP1-E	250	18.7	9.3	0.71	
	Deballast water of treated tank of the 3rd test run	S-C3-SP3-B1	200	2.4	1.5	0.09	0.08
		S-C3-SP3-B2	200	2.2	1.4	0.08	
		S-C3-SP3-B3	200	2.5	1.6	0.09	
		S-C3-SP3-M1	200	2.1	1.5	0.06	0.08
		S-C3-SP3-M2	200	2.3	1.6	0.07	
		S-C3-SP3-M3	200	2.5	1.6	0.09	
		S-C3-SP3-E1	200	2.6	1.7	0.09	0.08
		S-C3-SP3-E2	200	2.3	1.5	0.08	
		S-C3-SP3-E3	200	2.4	1.6	0.08	
2012.3.18	deballast water of control tank of the 3rd test run	S-C3-SP4-B	200	14.6	8.9	0.54	0.54
		S-C3-SP4-M	200	14.9	9.1	0.55	
		S-C3-SP4-E	200	14.6	9.0	0.53	

Analyst 李扬 Proofreader 孙华

Appendix 5 Results for microbes of the Shipboard Testing of Cyeco^{TM}-BWMS

Sampling date	Testing Run	Tank	Sample number	Intestinal *enterococci* (CFU/100mL)	*E.coli* (CFU/100mL)	*V.cholerae* (CFU/100mL)	Bacteria (CFU/100mL)
2011/10/25	Influent water of the 1st test run	control tank	S-C1-SP1--B/c	36	3.6×10^{2}	2.6×10^{3}	6.8×10^{4}
			S-C1-SP1-M/c	52	4.2×10^{2}	1.8×10^{3}	4.4×10^{4}
			S-C1-SP1-E/c	48	4.6×10^{2}	2.2×10^{3}	5.6×10^{4}
2011/10/26	deballast water of the 1st test run	treated tank	S-C1-SP3-B1/c	0	25	0	42
			S-C1-SP3-B2/c	0	22	0	28
			S-C1-SP3-B3/c	0	28	0	36
			S-C1-SP3-M1/c	0	13	0	28
			S-C1-SP3-M2/c	0	17	0	24
			S-C1-SP3-M3/c	0	19	0	31
			S-C1-SP3-E1/c	0	26	0	32
			S-C1-SP3-E2/c	0	19	0	18
			S-C1-SP3-E3/c	0	32	0	26

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Appendix 5 Results for microbes of the Shipboard Testing of Cyeco^{TM}-BWMS

Sampling date	Testing Run	Tank	Sample number	Intestinal *enterococci* (CFU/100mL)	*E.coli* (CFU/100mL)	*V.cholerae* (CFU/100mL)	Bacteria (CFU/100mL)
2011/10/26	Influent water of the 2nd test run	control tank	S-C2-SP1--B/c	1.9×10^{2}	1.7×10^{3}	1.3×10^{3}	2.4×10^{4}
			S-C2-SP1-M/c	2.2×10^{2}	1.9×10^{3}	1.6×10^{3}	2.6×10^{4}
			S-C2-SP1-E/c	1.8×10^{2}	1.5×10^{3}	1.1×10^{3}	2.2×10^{4}
2011/10/27	deballast water of the 2nd test run	treated tank	S-C2-SP3-B1/c	0	5	0	26
			S-C2-SP3-B2/c	0	0	0	22
			S-C2-SP3-B3/c	0	0	0	21
			S-C2-SP3-M1/c	0	0	0	25
			S-C2-SP3-M2/c	0	5	0	28
			S-C2-SP3-M3/c	0	0	0	31
			S-C2-SP3-E1/c	0	12	0	25
			S-C2-SP3-E2/c	0	36	0	18
			S-C2-SP3-E3/c	0	8	0	23

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Appendix 5 Results for microbes of the Shipboard Testing of Cyeco^{TM}-BWMS

Sampling date	Testing Run	Tank	Sample number	Intestinal *enterococci* (CFU/100mL)	*E.coli* (CFU/100mL)	*V.cholerae* (CFU/100mL)	Bacteria (CFU/100mL)
2012/3/17	Influent water of the 3rd test run	control tank	S-C3-SP1--B/c	0	60	1.3×10^3	3.6×10^4
			S-C3-SP1-M/c	0	90	1.5×10^3	5.6×10^4
			S-C3-SP1-E/c	0	100	1.7×10^3	5.2×10^4
2012/3/18	deballast water of the 3rd test run	treated tank	S-C3-SP3-B1/c	0	0	0	34
			S-C3-SP3-B2/c	0	0	0	36
			S-C3-SP3-B3/c	0	0	0	30
			S-C3-SP3-M1/c	0	0	0	25
			S-C3-SP3-M2/c	0	0	0	20
			S-C3-SP3-M3/c	0	0	0	20
			S-C3-SP3-E1/c	0	0	0	0
			S-C3-SP3-E2/c	0	0	0	0
			S-C3-SP3-E3/c	0	0	0	0

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